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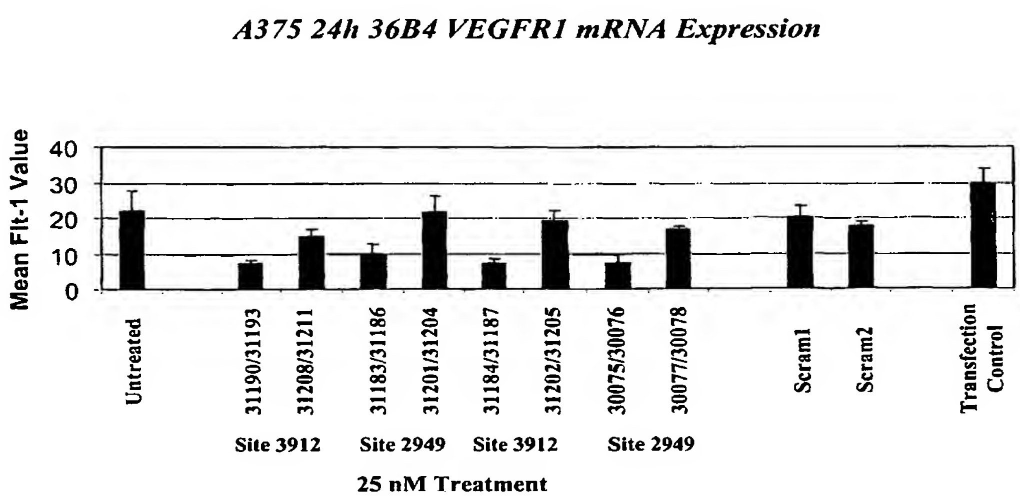
(54) Title: RNA INTERFERENCE MEDIATED INHIBITION OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR GENE EXPRESSION USING SHORT INTERFERING NUCLEIC ACID (siNA)



(57) Abstract: The present invention concerns methods and reagents useful in modulating vascular endothelial growth factor (VEGF, VEGF-B, VEGF-C, VEGF-D) and/or vascular endothelial growth factor receptor (e.g., VEGFr1, VEGFr2, and/or VEGFr3) gene expression in a variety of applications, including use in therapeutic, diagnostic, target validation, and genomic discovery applications. Specifically, the invention relates to small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi) against VEGF and/or VEGFr gene expression and/or activity. The small nucleic acid molecules are useful in the diagnosis and treatment of cancer, proliferative diseases, and any other disease or condition that responds to modulation of VEGF and/or VEGFr expression or activity.

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(71) Applicant *(for all designated States except US)*: **RIBOZYME PHARMACEUTICALS, INCORPORATED** [US/US]; 2950 Wilderness Place, Boulder, CO 80301 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **McSWIGGEN, James** [US/US]; 4866 Franklin Drive, Boulder, CO 80301 (US). **BEIGELMAN, Leonid** [US/US]; 5530 Colt Drive, Longmont, CO 80503 (US). **PAVCO, Pamela** [US/US]; 705 Barberry Circle, Lafayette, CO 80026 (US).

(74) Agent: TERPSTRA, Anita, J.; McDonnell Boehnen Hulbert & Berghoff, Suite 3200, 300 South Wacker Drive, Chicago, IL 60606 (US).

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RNA INTERFERENCE MEDIATED INHIBITION OF VASCULAR EDOTHELIAL GROWTH FACTOR AND VASCULAR EDOTHELIAL GROWTH FACTOR RECEPTOR GENE EXPRESSION USING SHORT INTERFERING NUCLEIC ACID (siNA)

This invention claims the benefit of McSwiggen, USSN 60/393,796 filed July 3, 2002, of McSwiggen, USSN 60/399,348 filed July 29, 2002, of Pavco, USSN 10/306,747, filed November 27, 2002, which claims the benefit of Pavco USSN 60/334461, filed November 30, 2001, of Pavco, USSN 10/287,949 filed November 4, 2002, of Pavco, PCT/US02/17674 filed May 29, 2002, of Beigelman USSN 60/358,580 filed February 20, 2002, of Beigelman USSN 60/363,124 filed March 11, 2002, of Beigelman USSN 60/386,782 filed June 6, 2002, of Beigelman USSN 60/406,784 filed August 29,2002, of Beigelman USSN 60/408,378 filed September 5, 2002, of Beigelman USSN 60/409,293 filed September 9, 2002, and of Beigelman USSN 60/440,129 filed January 15, 2003. These applications àre hereby incorporated by reference herein in their entireties, including the drawings.

Field Of The Invention

The present invention concerns compounds, compositions, and methods for the study, diagnosis, and treatment of conditions and diseases that respond to the modulation of vascular endothelial growth factor (VEGF) and/or vascular endothelial growth factor receptor (e.g., VEGFr1, VEGFr2 and/or VEGFr3) gene expression and/or activity. The present invention also concerns compounds, compositions, and methods relating to conditions and diseases that respond to the modulation of expression and/or activity of genes involved in VEGF and VEGF receptor pathways. Specifically, the invention relates to small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi) against VEGF and VEGF receptor gene expression.

Background Of The Invention

The following is a discussion of relevant art pertaining to RNAi. The discussion is provided only for understanding of the invention that follows. The summary is not an admission that any of the work described below is prior art to the claimed invention.

RNA interference refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire *et al.*, 1998, *Nature*, 391, 806). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response though a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Berstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having

sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188).

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature*, 391, 806, were the first to observe RNAi in *C. elegans*. Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mouse embryos. Hammond *et al.*, 2000, *Nature*, 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.*, 2001, *Nature*, 411, 494, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in Drosophila embryonic lysates (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877) has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21-nucleotide siRNA duplexes are most active when containing 3'-terminal dinucleotide overhangs. Furthermore, complete substitution of one or both siRNA strands with 2'-deoxy (2'-H) or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of the 3'-terminal siRNA overhang nucleotides with 2'-deoxy nucleotides (2'-H) was shown to be tolerated. Single mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end of the guide sequence (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.*, 2001, *Cell*, 107, 309).

Studies have shown that replacing the 3'-terminal nucleotide overhanging segments of a 21-mer siRNA duplex having two -nucleotide 3'-overhangs with deoxyribonucleotides does not have an adverse effect on RNAi activity. Replacing up to four nucleotides on each end of the siRNA with deoxyribonucleotides has been reported to be well tolerated, whereas complete substitution with deoxyribonucleotides results in no RNAi activity (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). In addition, Elbashir *et al.*, *supra*, also report that substitution of siRNA with 2'-O-methyl nucleotides completely abolishes RNAi activity. Li *et al.*,

International PCT Publication No. WO 00/44914, and Beach *et al.*, International PCT Publication No. WO 01/68836 preliminarily suggest that siRNA may include modifications to either the phosphate-sugar backbone or the nucleoside to include at least one of a nitrogen or sulfur heteroatom, however, neither application postulates to what extent such modifications would be tolerated in siRNA molecules, nor provides any further guidance or examples of such modified siRNA. Kreutzer *et al.*, Canadian Patent Application No. 2,359,180, also describe certain chemical modifications for use in dsRNA constructs in order to counteract activation of double-stranded RNA-dependent protein kinase PKR, specifically 2'-amino or 2'-O-methyl nucleotides, and nucleotides containing a 2'-O or 4'-C methylene bridge. However, Kreutzer *et al.* similarly fails to provide examples or guidance as to what extent these modifications would be tolerated in siRNA molecules.

Parrish *et al.*, 2000, *Molecular Cell*, 6, 1977-1087, tested certain chemical modifications targeting the unc-22 gene in *C. elegans* using long (>25 nt) siRNA transcripts. The authors describe the introduction of thiophosphate residues into these siRNA transcripts by incorporating thiophosphate nucleotide analogs with T7 and T3 RNA polymerase and observed that RNAs with two phosphorothioate modified bases also had substantial decreases in effectiveness as RNAi. Further, Parrish *et al.* reported that phosphorothioate modification of more than two residues greatly destabilized the RNAs *in vitro* such that interference activities could not be assayed. *Id.* at 1081. The authors also tested certain modifications at the 2'-position of the nucleotide sugar in the long siRNA transcripts and found that substituting deoxynucleotides for ribonucleotides produced a substantial decrease in interference activity, especially in the case of Uridine to Thymidine and/or Cytidine to deoxy-Cytidine substitutions. *Id.* In addition, the authors tested certain base modifications, including substituting, in sense and antisense strands of the siRNA, 4-thiouracil, 5-bromouracil, 5-iodouracil, and 3-(aminoallyl)uracil for uracil, and inosine for guanosine. Whereas 4-thiouracil and 5-bromouracil substitution appeared to be tolerated, Parrish reported that inosine produced a substantial decrease in interference activity when incorporated in either strand. Parrish also reported that incorporation of 5-iodouracil and 3-(aminoallyl)uracil in the antisense strand resulted in a substantial decrease in RNAi activity as well.

The use of longer dsRNA has been described. For example, Beach *et al.*, International PCT Publication No. WO 01/68836, describes specific methods for attenuating gene expression using endogenously-derived dsRNA. Tuschl *et al.*, International PCT Publication No. WO 01/75164, describe a *Drosophila in vitro* RNAi system and the use of specific siRNA molecules for certain functional genomic and certain therapeutic applications; although Tuschl, 2001, *Chem. Biochem.*, 2, 239-245, doubts that RNAi can be used to cure genetic diseases or viral infection due to the danger of activating interferon response. Li *et al.*, International PCT Publication No. WO 00/44914, describe the use of specific dsRNAs for attenuating the expression of certain target genes. Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646, describe certain methods for inhibiting the expression of particular genes in mammalian cells using certain dsRNA molecules. Fire *et al.*, International PCT Publication No. WO 99/32619, describe particular methods for introducing certain dsRNA molecules into cells for use in inhibiting gene expression. Plaetinck *et al.*, International PCT Publication No. WO 00/01846, describe certain methods for identifying specific genes responsible for conferring a particular phenotype in a cell using specific dsRNA molecules. Mello *et al.*, International PCT Publication No. WO 01/29058, describe the identification of specific genes involved in dsRNA-mediated RNAi. Deschamps Depaillette *et al.*, International PCT Publication No. WO 99/07409, describe specific compositions consisting of particular dsRNA molecules combined with certain anti-viral agents. Waterhouse *et al.*, International PCT Publication No. 99/53050, describe certain methods for decreasing the phenotypic expression of a nucleic acid in plant cells using certain dsRNAs. Driscoll *et al.*, International PCT Publication No. WO 01/49844, describe specific DNA constructs for use in facilitating gene silencing in targeted organisms.

Others have reported on various RNAi and gene-silencing systems. For example, Parrish *et al.*, 2000, *Molecular Cell*, 6, 1977-1087, describe specific chemically-modified siRNA constructs targeting the unc-22 gene of *C. elegans*. Grossniklaus, International PCT Publication No. WO 01/38551, describes certain methods for regulating polycomb gene expression in plants using certain dsRNAs. Churikov *et al.*, International PCT Publication No. WO 01/42443, describe certain methods for modifying genetic characteristics of an organism using certain dsRNAs. Cogoni *et al.*, International PCT Publication No. WO

01/53475, describe certain methods for isolating a Neurospora silencing gene and uses thereof. Reed *et al.*, International PCT Publication No. WO 01/68836, describe certain methods for gene silencing in plants. Honer *et al.*, International PCT Publication No. WO 01/70944, describe certain methods of drug screening using transgenic nematodes as Parkinson's Disease models using certain dsRNAs. Deak *et al.*, International PCT Publication No. WO 01/72774, describe certain *Drosophila*-derived gene products that may be related to RNAi .in *Drosophila*. Arndt *et al.*, International PCT Publication No. WO 01/92513 describe certain methods for mediating gene suppression by using factors that enhance RNAi. Tuschl *et al.*, International PCT Publication No. WO 02/44321, describe certain synthetic siRNA constructs. Pachuk *et al.*, International PCT Publication No. WO 00/63364, and Satishchandran *et al.*, International PCT Publication No. WO 01/04313, describe certain methods and compositions for inhibiting the function of certain polynucleotide sequences using certain dsRNAs. Echeverri *et al.*, International PCT Publication No. WO 02/38805, describe certain *C. elegans* genes identified via RNAi. Kreutzer *et al.*, International PCT Publications Nos. WO 02/055692, WO 02/055693, and EP 1144623 B1 describes certain methods for inhibiting gene expression using RNAi. Graham *et al.*, International PCT Publications Nos. WO 99/49029 and WO 01/70949, and AU 4037501 describe certain vector expressed siRNA molecules. Fire *et al.*, US 6,506,559, describe certain methods for inhibiting gene expression in vitro using certain long dsRNA (greater than 25 nucleotide) constructs that mediate RNAi.

SUMMARY OF THE INVENTION

This invention relates to compounds, compositions, and methods useful for modulating the expression of genes, such as those genes associated with angiogenesis and proliferation using short interfering nucleic acid (siNA) molecules. This invention also relates to compounds, compositions, and methods useful for modulating the expression and activity of vascular endothelial growth factor (VEGF) and/or vascular endothelial growth factor receptor (e.g., VEGFr1, VEGFr2, VEGFr3) genes, or genes involved in VEGF and/or VEGFr pathways of gene expression and/or VEGF activity by RNA interference (RNAi) using small nucleic acid molecules, such as short interfering nucleic acid (siNA), short

interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules. In particular, the instant invention features small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules and methods used to modulate the expression of VEGF and/or VEGFr genes. A siNA of the invention can be unmodified or chemically-modified. A siNA of the instant invention can be chemically synthesized, expressed from a vector or enzymatically synthesized. The instant invention also features various chemically-modified synthetic short interfering nucleic acid (siNA) molecules capable of modulating VEGF and/or VEGFr gene expression or activity in cells by RNA interference (RNAi). The use of chemically-modified siNA improves various properties of native siNA molecules through increased resistance to nuclease degradation *in vivo* and/or through improved cellular uptake. Further, contrary to earlier published studies, siNA having multiple chemical modifications retains its RNAi activity. The siNA molecules of the instant invention provide useful reagents and methods for a variety of therapeutic, diagnostic, target validation, genomic discovery, genetic engineering, and pharmacogenomic applications.

In one embodiment, the invention features one or more siNA molecules and methods that independently or in combination modulate the expression of gene(s) encoding proteins, such as vascular endothelial growth factor (VEGF) and/or vascular endothelial growth factor receptors (e.g., VEGFr1, VEGFr2, VEGFr3), associated with the maintenance and/or development of cancer and other proliferative diseases, such as genes encoding sequences comprising those sequences referred to by GenBank Accession Nos. shown in **Table I**, referred to herein generally as VEGF and/or VEGFr. The description below of the various aspects and embodiments of the invention is provided with reference to the exemplary VEGF and VEGFr (e.g., VEGFr1, VEGFr2, VEGFr3) genes referred to herein as VEGF and VEGFr respectively. However, the various aspects and embodiments are also directed to other VEGF and/or VEGFr genes, such as mutant VEGF and/or VEGFr genes, splice variants of VEGF and/or VEGFr genes, other VEGF and/or VEGFr ligands and receptors. The various aspects and embodiments are also directed to other genes that are involved in VEGF and/or VEGFr mediated pathways of signal transduction or gene expression that are

involved in the progression, development, and/or maintenance of disease (e.g., cancer). Those additional genes can be analyzed for target sites using the methods described for VEGF and/or VEGFr genes herein. Thus, the inhibition and the effects of such inhibition of the other genes can be performed as described herein.

In one embodiment, the invention features a siNA molecule that down-regulates expression of a VEGF gene, for example, wherein the VEGF gene comprises VEGF encoding sequence.

In one embodiment, the invention features a siNA molecule that down-regulates expression of a VEGFr gene, for example, wherein the VEGFr gene comprises VEGFr encoding sequence.

In one embodiment, the invention features a siNA molecule having RNAi activity against VEGF and/or VEGFr RNA, wherein the siNA molecule comprises a sequence complementary to any RNA having VEGF and/or VEGFr or other VEGF and/or VEGFr encoding sequence, such as those sequences having GenBank Accession Nos. shown in **Table I**. Chemical modifications as shown in **Tables III and IV** or otherwise described herein can be applied to any siNA construct of the invention.

In one embodiment, the invention features a siNA molecule having RNAi activity against VEGF and/or VEGFr RNA, wherein the siNA molecule comprises a sequence complementary to any RNA having VEGF and/or VEGFr encoding sequence, such as those sequences having VEGF and/or VEGFr GenBank Accession Nos. shown in **Table I**. Chemical modifications as shown in **Tables III and IV** or otherwise described herein can be applied to any siNA construct of the invention.

In another embodiment, the invention features a siNA molecule having RNAi activity against a VEGF and/or VEGFr gene, wherein the siNA molecule comprises nucleotide sequence complementary to nucleotide sequence of a VEGF and/or VEGFr gene, such as those VEGF and/or VEGFr sequences having GenBank Accession Nos. shown in **Table I**. In another embodiment, a siNA molecule of the invention includes nucleotide sequence that can interact with nucleotide sequence of a VEGF and/or VEGFr gene and thereby mediate

silencing of VEGF and/or VEGFr gene expression, for example, wherein the siNA mediates regulation of VEGF and/or VEGFr gene expression by cellular processes that modulate the chromatin structure of the VEGF and/or VEGFr gene and prevent transcription of the VEGF and/or VEGFr gene.

In another embodiment, the invention features a siNA molecule comprising nucleotide sequence, for example, nucleotide sequence in the antisense region of the siNA molecule that is complementary to a nucleotide sequence or portion of sequence of a VEGF and/or VEGFr gene. In another embodiment, the invention features a siNA molecule comprising a region, for example, the antisense region of the siNA construct, complementary to a sequence or portion of sequence comprising a VEGF and/or VEGFr gene sequence.

In one embodiment, the antisense region of VEGFr1 siNA constructs can comprise a sequence complementary to sequence having any of SEQ ID NOs. 1-427 or 1997-2000. In one embodiment, the antisense region can also comprise sequence having any of SEQ ID NOs. 428-854, 2024-2027, 2032-2035, 2040-2043, 2104-2107, 2109, 2117, 2120-2122, 2125-2132, 2137-2140, 2142, 2150, 2152, 2154, 2158-2160, 2164-2166, 2188-2190, 2197, 2199, 2203-2204, 2229, 2231, 2233, 2235, 2237, or 2238. In another embodiment, the sense region of VEGFr1 constructs can comprise sequence having any of SEQ ID NOs. 1-427, 1997-2000, 2009-2016, 2020-2023, 2028-2031, 2036-2039, 2092-2103, 2108, 2114, 2116, 2123-2124, 2133-2136, 2141, 2149, 2151, 2153, 2155-2157, 2161-2163, 2185-2187, 2198, 2200-2202, 2228, 2230, 2232, 2234, or 2236. The sense region can comprise a sequence of SEQ ID NO. 2217 and the antisense region can comprise a sequence of SEQ ID NO. 2218. The sense region can comprise a sequence of SEQ ID NO. 2219 and the antisense region can comprise a sequence of SEQ ID NO. 2220. The sense region can comprise a sequence of SEQ ID NO. 2221 and the antisense region can comprise a sequence of SEQ ID NO. 2222. The sense region can comprise a sequence of SEQ ID NO. 2223 and the antisense region can comprise a sequence of SEQ ID NO. 2224. The sense region can comprise a sequence of SEQ ID NO. 2225 and the antisense region can comprise a sequence of SEQ ID NO. 2226. The sense region can comprise a sequence of SEQ ID NO. 2223 and the antisense region can comprise a sequence of SEQ ID NO. 2227.

In one embodiment, the antisense region of VEGFr2 siNA constructs can comprise a sequence complementary to sequence having any of SEQ ID NOs. 855-1178 or 2001-2004. In one embodiment, the antisense region can also comprise sequence having any of SEQ ID NOs. 1179-1502, 2048-2051, 2056-2059, 2064-2067, 2208-2210, 2214-2216, or 2048-2051. In another embodiment, the sense region of VEGFr2 constructs can comprise sequence having any of SEQ ID NOs. 855-1178, 2001-2004, 2044-2047, 2052-2055, 2060-2063, 2017-2019, 2205-2207, 2211-2213, or 2044-2047. The sense region can comprise a sequence of SEQ ID NO. 2217 and the antisense region can comprise a sequence of SEQ ID NO. 2218. The sense region can comprise a sequence of SEQ ID NO. 2219 and the antisense region can comprise a sequence of SEQ ID NO. 2220. The sense region can comprise a sequence of SEQ ID NO. 2221 and the antisense region can comprise a sequence of SEQ ID NO. 2222. The sense region can comprise a sequence of SEQ ID NO. 2223 and the antisense region can comprise a sequence of SEQ ID NO. 2224. The sense region can comprise a sequence of SEQ ID NO. 2225 and the antisense region can comprise a sequence of SEQ ID NO. 2226. The sense region can comprise a sequence of SEQ ID NO. 2223 and the antisense region can comprise a sequence of SEQ ID NO. 2227.

In one embodiment, the antisense region of VEGFr3 siNA constructs can comprise a sequence complementary to sequence having any of SEQ ID NOs. 1503-1749 or 2005-2008. In one embodiment, the antisense region can also comprise sequence having any of SEQ ID NOs. 1750-1996, 2072-2075, 2080-2083, or 2088-2091. In another embodiment, the sense region of VEGFr3 constructs can comprise sequence having any of SEQ ID NOs. 1503-1749, 2005-2008, 2068-2071, 2076-2079, or 2034-2087. The sense region can comprise a sequence of SEQ ID NO. 2217 and the antisense region can comprise a sequence of SEQ ID NO. 2218. The sense region can comprise a sequence of SEQ ID NO. 2219 and the antisense region can comprise a sequence of SEQ ID NO. 2220. The sense region can comprise a sequence of SEQ ID NO. 2221 and the antisense region can comprise a sequence of SEQ ID NO. 2222. The sense region can comprise a sequence of SEQ ID NO. 2223 and the antisense region can comprise a sequence of SEQ ID NO. 2224. The sense region can comprise a sequence of SEQ ID NO. 2225 and the antisense region can comprise a sequence

of SEQ ID NO. 2226. The sense region can comprise a sequence of SEQ ID NO. 2223 and the antisense region can comprise a sequence of SEQ ID NO. 2227.

In one embodiment, a siNA molecule of the invention comprises any of SEQ ID NOs. 1-2238. The sequences shown in SEQ ID NOs: 1-2238 are not limiting. A siNA molecule of the invention can comprise any contiguous VEGF and/or VEGFr sequence (e.g., about 19 to about 25, or about 19, 20, 21, 22, 23, 24 or 25 contiguous VEGF and/or VEGFr nucleotides).

In yet another embodiment, the invention features a siNA molecule comprising a sequence, for example, the antisense sequence of the siNA construct, complementary to a sequence or portion of sequence comprising sequence represented by GenBank Accession Nos. shown in **Table I**. Chemical modifications in **Tables III and IV** and descrbed herein can be applied to any siRNA costruct of the invention.

In one embodiment of the invention a siNA molecule comprises an antisense strand having about 19 to about 29 nucleotides, wherein the antisense strand is complementary to a RNA sequence encoding a VEGF and/or VEGFr protein, and wherein said siNA further comprises a sense strand having about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29) nucleotides, and wherein said sense strand and said antisense strand are distinct nucleotide sequences with at least about 19 complementary nucleotides.

In another embodiment of the invention a siNA molecule of the invention comprises an antisense region having about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29) nucleotides, wherein the antisense region is complementary to a RNA sequence encoding a VEGF and/or VEGFr protein, and wherein said siNA further comprises a sense region having about 19 to about 29 nucleotides, wherein said sense region and said antisense region comprise a linear molecule with at least about 19 complementary nucleotides.

In one embodiment of the invention a siNA molecule comprises an antisense strand comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof encoding a VEGF and/or VEGFr protein. The siNA further comprises a

sense strand, wherein said sense strand comprises a nucleotide sequence of a VEGF and/or VEGFr gene or a portion thereof.

In another embodiment, a siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof encoding a VEGF and/or VEGFr protein. The siNA molecule further comprises a sense region, wherein said sense region comprises a nucleotide sequence of a VEGF and/or VEGFr gene or a portion thereof.

In one embodiment, a siNA molecule of the invention has RNAi activity that modulates expression of RNA encoded by a VEGF gene. Because VEGF genes can share some degree of sequence homology with each other, siNA molecules can be designed to target a class of VEGF genes (and associated receptor or ligand genes) or alternately specific VEGF genes by selecting sequences that are either shared amongst different VEGF targets or alternatively that are unique for a specific VEGF target. Therefore, in one embodiment, the siNA molecule can be designed to target conserved regions of VEGF RNA sequence having homology between several VEGF genes so as to target several VEGF genes (e.g., different VEGF isoforms, splice variants, mutant genes etc.) with one siNA molecule. In another embodiment, the siNA molecule can be designed to target a sequence that is unique to a specific VEGF RNA sequence due to the high degree of specificity that the siNA molecule requires to mediate RNAi activity.

In one embodiment, a siNA molecule of the invention has RNAi activity that modulates expression of RNA encoded by a VEGFr gene. Because VEGFr genes can share some degree of sequence homology with each other, siNA molecules can be designed to target a class of VEGFr genes (and associated receptor or ligand genes) or alternately specific VEGFr genes by selecting sequences that are either shared amongst different VEGFr targets or alternatively that are unique for a specific VEGFr target. Therefore, in one embodiment, the siNA molecule can be designed to target conserved regions of VEGFr RNA sequence having homology between several VEGFr genes so as to target several VEGFr genes (e.g., different VEGFr isoforms, splice variants, mutant genes etc.) with one siNA molecule. In another embodiment, the siNA molecule can be designed to target a

sequence that is unique to a specific VEGFr RNA sequence due to the high degree of specificity that the siNA molecule requires to mediate RNAi activity.

In one embodiment, a siNA molecule of the invention has RNAi activity that modulates expression of RNA encoded by a VEGFr gene. Because VEGFr genes can share some degree of sequence homology with each other, siNA molecules can be designed to target a class of VEGFr genes or alternately specific VEGFr genes by selecting sequences that are either shared amongst different VEGFr targets or alternatively that are unique for a specific VEGFr target. Therefore, in one embodiment, the siNA molecule can be designed to target conserved regions of VEGFr RNA sequence having homology between several VEGFr genes so as to target several VEGFr genes (e.g., VEGFr1, VEGFr2 and/or VEGFr3, different VEGFr isoforms, splice variants, mutant genes etc.) with one siNA molecule. In another embodiment, the siNA molecule can be designed to target a sequence that is unique to a specific VEGFr RNA sequence due to the high degree of specificity that the siNA molecule requires to mediate RNAi activity.

In one embodiment, a siNA molecule of the invention has RNAi activity that modulates expression of RNA encoded by a VEGF gene. Because VEGF genes can share some degree of sequence homology with each other, siNA molecules can be designed to target a class of VEGF genes or alternately specific VEGF genes by selecting sequences that are either shared amongst different VEGF targets or alternatively that are unique for a specific VEGF target. Therefore, in one embodiment, the siNA molecule can be designed to target conserved regions of VEGF RNA sequence having homology between several VEGF genes so as to target several VEGF genes (e.g., VEGF-A, VEGF-B, VEGF-C and/or VEGF-D, different VEGF isoforms, splice variants, mutant genes etc.) with one siNA molecule. In another embodiment, the siNA molecule can be designed to target a sequence that is unique to a specific VEGF RNA sequence due to the high degree of specificity that the siNA molecule requires to mediate RNAi activity.

In one embodiment, nucleic acid molecules of the invention that act as mediators of the RNA interference gene silencing response are double-stranded nucleic acid molecules. In another embodiment, the siNA molecules of the invention consist of duplexes containing

about 19 base pairs between oligonucleotides comprising about 19 to about 25 (*e.g.*, about 19, 20, 21, 22, 23, 24 or 25) nucleotides. In yet another embodiment, siNA molecules of the invention comprise duplexes with overhanging ends of about about 1 to about 3 (*e.g.*, about 1, 2, or 3) nucleotides, for example, about 21-nucleotide duplexes with about 19 base pairs and 3'-terminal mononucleotide, dinucleotide, or trinucleotide overhangs.

In one embodiment, the invention features one or more chemically-modified siNA constructs having specificity for VEGF and/or VEGFr expressing nucleic acid molecules, such as RNA encoding a VEGF and/or VEGFr protein. Non-limiting examples of such chemical modifications include without limitation phosphorothioate internucleotide linkages, 2'-deoxyribonucleotides, 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, "universal base" nucleotides, "acyclic" nucleotides, 5-C-methyl nucleotides, and terminal glyceryl and/or inverted deoxy abasic residue incorporation. These chemical modifications, when used in various siNA constructs, are shown to preserve RNAi activity in cells while at the same time, dramatically increasing the serum stability of these compounds. Furthermore, contrary to the data published by Parrish *et al., supra,* applicant demonstrates that multiple (greater than one) phosphorothioate substitutions are well-tolerated and confer substantial increases in serum stability for modified siNA constructs.

In one embodiment, a siNA molecule of the invention comprises modified nucleotides while maintaining the ability to mediate RNAi. The modified nucleotides can be used to improve *in vitro* or *in vivo* characteristics such as stability, activity, and/or bioavailability. For example, a siNA molecule of the invention can comprise modified nucleotides as a percentage of the total number of nucleotides present in the siNA molecule. As such, a siNA molecule of the invention can generally comprise about 5% to about 100% modified nucleotides (e.g., 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides). The actual percentage of modified nucleotides present in a given siNA molecule will depend on the total number of nucleotides present in the siNA. If the siNA molecule is single stranded, the percent modification can be based upon the total number of nucleotides present in the single stranded siNA molecules. Likewise, if the siNA molecule is double stranded, the percent

modification can be based upon the total number of nucleotides present in the sense strand, antisense strand, or both the sense and antisense strands.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene, wherein the siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is about 21 nucleotides long.

In one embodiment, a siNA molecule of the invention comprises no ribonucleotides. In another embodiment, a siNA molecule of the invention comprises ribonucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the VEGF and/or VEGFr gene, and wherein the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of the VEGF and/or VEGFr gene.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene, wherein each strand of the siNA molecule comprises about 19 to about 23 nucleotides, and wherein each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene, wherein the siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the VEGF and/or VEGFr gene, and wherein the siNA further comprises a sense region, wherein the sense region comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of the VEGF and/or VEGFr gene.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene, wherein the antisense region and the sense region each comprise about 19 to about 23 nucleotides, and wherein the antisense region comprises at least about 19 nucleotides that are complementary to nucleotides of the sense region.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene, wherein the siNA molecule comprises a sense region and an antisense region and wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of RNA encoded by the VEGF and/or VEGFr gene and the sense region comprises a nucleotide sequence that is complementary to the antisense region.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. The sense region can be connected to the antisense region via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene, wherein the siNA molecule comprises a sense region and an antisense region and wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of RNA encoded by the VEGF and/or VEGFr gene and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein pyrimidine nucleotides in the sense region are 2'-O-methyl pyrimidine nucleotides, 2'-deoxy purine nucleotides, or 2'-deoxy-2'-fluoro pyrimidine nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments

wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule, and wherein the fragment comprising the sense region includes a terminal cap moiety at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the fragment comprising the sense region. In another embodiment, the terminal cap moiety is an inverted deoxy abasic moiety or glyceryl moiety. In another embodiment, each of the two fragments of the siNA molecule comprise about 21 nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene, wherein the siNA molecule comprises a sense region and an antisense region and wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of RNA encoded by the VEGF and/or VEGFr gene and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein the purine nucleotides present in the antisense region comprise 2'-deoxy- purine nucleotides. In another embodiment, the antisense region comprises a phosphorothioate internucleotide linkage at the 3' end of the antisense region. In another embodiment, the antisense region comprises a glyceryl modification at the 3' end of the antisense region.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a VEGF and/or VEGFr gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule, and wherein about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule and wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule. In another embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule are 2'-deoxy-pyrimidines, such as 2'-deoxy-thymidine. In another embodiment, all 21 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule. In another embodiment, about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the VEGF and/or VEGFr

gene. In another embodiment, 21 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the VEGF and/or VEGFr gene. In another embodiment, the 5'-end of the fragment comprising said antisense region optionally includes a phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a VEGF and/or VEGFr RNA sequence (e.g., wherein said target RNA sequence is encoded by a VEGF and/or VEGFr gene), wherein the siNA molecule comprises no ribonucleotides and wherein each strand of the double-stranded siNA molecule is about 21 nucleotides long.

In one embodiment, the invention features a medicament comprising a siNA molecule of the invention.

In one embodiment, the invention features an active ingredient comprising a siNA molecule of the invention.

In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule to down-regulate expression of a VEGF and/or VEGFr gene, wherein the siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is about 21 nucleotides long.

In one embodiment, a VEGFr gene contemplated by the invention is a VEGFr1, VEGFr2, or VEGFr3 gene.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule

comprises a sugar modification. In one embodiment, the VEGFr gene is VEGFr2. In one embodiment, the VEGFr gene is VEGFr1.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence of the antisense strand of the double-stranded siNA molecule is complementary to the nucleotide sequence of the VEGF and/or VEGFr RNA or a portion thereof which encodes an protein or a portion thereof.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein each strand of the siNA molecule comprises about 19 to about 29 nucleotides, and wherein each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein

a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the siNA molecule is assembled from two oligonucleotide fragments wherein one fragment comprises the nucleotide sequence of the antisense strand of the siNA moleculeand a second fragment comprises nucleotide sequence of the sense region of the siNA molecule.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the sense strand is connected to the antisense strand via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and wherein purine nucleotides present in the sense region are 2'-deoxy purine nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide

sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the sense strand comprises a 3'-end and a 5'-end, and wherein a terminal cap moiety (e.g., an inverted deoxy abasic moiety) is present at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the sense strand.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the antisense strand comprises one or more 2'-deoxy-2'-fluoro pyrimidine nucleotides and one or more 2'-O-methyl purine nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and wherein any purine nucleotides present in the antisense strand are 2'-O-methyl purine nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide

sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the antisense strand comprises a phosphorothioate internucleotide linkage at the 3' end of the antisense strand.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the antisense strand comprises a glyceryl modification at the 3' end.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein each of the two strands of the siNA molecule comprises 21 nucleotides. In another embodiment, about 19 nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule and wherein at least two 3' terminal nucleotides of each strand of the siNA molecule are not base-paired to the nucleotides of the other strand of the siNA molecule. In another embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule are 2'-deoxy-pyrimidines, such as 2'-deoxy-thymidine. In another embodiment, each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule. In another embodiment, about 19 nucleotides of the antisense strand are base-paired to the nucleotide sequence of the VEGF

and/or VEGFr RNA or a portion thereof. In another embodiment, 21 nucleotides of the antisense strand are base-paired to the nucleotide sequence of the VEGF and/or VEGFr RNA or a portion thereof.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the 5'-end of the antisense strand optionally includes a phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence or a portion thereof of the antisense strand is complementary to a nucleotide sequence of the 5'-untranslated region or a portion thereof of the VEGF and/or VEGFr RNA.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule

comprises a sugar modification, and wherein the nucleotide sequence or a portion thereof of the antisense strand is complementary to a nucleotide sequence of the VEGF and/or VEGFr RNA or a portion thereof that is present in the VEGF and/or VEGFr RNA.

In one embodiment, the invention features a pharmaceutical composition comprising a siNA molecule of the invention in an acceptable carrier or diluent.

In one embodiment, the invention features a medicament comprising an siNA molecule of the invention.

In one embodiment, the invention features an active ingredient comprising an siNA molecule of the invention.

In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a VEGF and/or VEGFr gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of VEGF and/or VEGFr RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

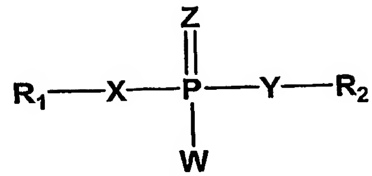
In a non-limiting example, the introduction of chemically-modified nucleotides into nucleic acid molecules provides a powerful tool in overcoming potential limitations of *in vivo* stability and bioavailability inherent to native RNA molecules that are delivered exogenously. For example, the use of chemically-modified nucleic acid molecules can enable a lower dose of a particular nucleic acid molecule for a given therapeutic effect since chemically-modified nucleic acid molecules tend to have a longer half-life in serum. Furthermore, certain chemical modifications can improve the bioavailability of nucleic acid molecules by targeting particular cells or tissues and/or improving cellular uptake of the nucleic acid molecule. Therefore, even if the activity of a chemically-modified nucleic acid molecule is reduced as compared to a native nucleic acid molecule, for example, when compared to an all-RNA nucleic acid molecule, the overall activity of the modified nucleic

acid molecule can be greater than that of the native molecule due to improved stability and/or delivery of the molecule. Unlike native unmodified siNA, chemically-modified siNA can also minimize the possibility of activating interferon activity in humans.

The antisense region of a siNA molecule of the invention can comprise a phosphorothioate internucleotide linkage at the 3'-end of said antisense region. The antisense region can comprise about one to about five phosphorothioate internucleotide linkages at the 5'-end of said antisense region. The 3'-terminal nucleotide overhangs of a siNA molecule of the invention can comprise ribonucleotides or deoxyribonucleotides that are chemically-modified at a nucleic acid sugar, base, or backbone. The 3'-terminal nucleotide overhangs can comprise one or more universal base ribonucleotides. The 3'-terminal nucleotide overhangs can comprise one or more acyclic nucleotides.

One embodiment of the invention provides an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention in a manner that allows expression of the nucleic acid molecule. Another embodiment of the invention provides a mammalian cell comprising such an expression vector. The mammalian cell can be a human cell. The siNA molecule of the expression vector can comprise a sense region and an antisense region. The antisense region can comprise sequence complementary to a RNA or DNA sequence encoding VEGF and/or VEGFr and the sense region can comprise sequence complementary to the antisense region. The siNA molecule can comprise two distinct strands having complementary sense and antisense regions. The siNA molecule can comprise a single strand having complementary sense and antisense regions.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides comprising a backbone modified internucleotide linkage having Formula I:

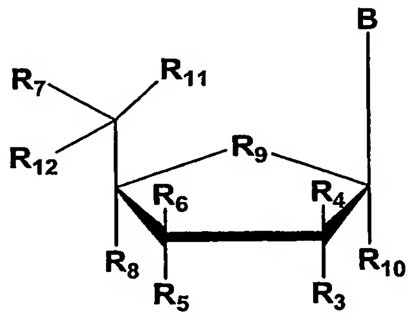


wherein each R1 and R2 is independently any nucleotide, non-nucleotide, or polynucleotide which can be naturally-occurring or chemically-modified, each X and Y is independently O, S, N, alkyl, or substituted alkyl, each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl, and wherein W, X, Y, and Z are optionally not all O.

The chemically-modified internucleotide linkages having Formula I, for example, wherein any Z, W, X, and/or Y independently comprises a sulphur atom, can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) chemically-modified internucleotide linkages having Formula I at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified internucleotide linkages having Formula I at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In another embodiment, a siNA molecule of the invention having internucleotide linkage(s) of Formula I also comprises a chemically-modified nucleotide or non-nucleotide having any of Formulae I-VII.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a

VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula II:

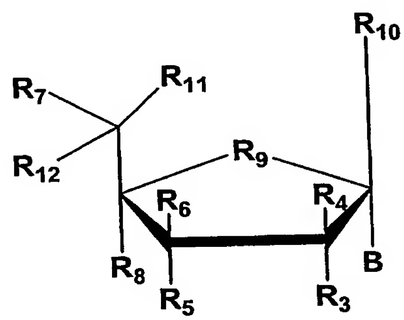


wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I; R9 is O, S, CH2, S=O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

The chemically-modified nucleotide or non-nucleotide of Formula II can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotide or non-nucleotide of Formula II at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 5'-end of the sense strand, the antisense strand, or both

strands. In anther non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 3'-end of the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula III:

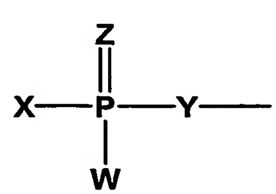


wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I; R9 is O, S, CH2, S=O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be employed to be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

The chemically-modified nucleotide or non-nucleotide of Formula III can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide(s) or non-nucleotide(s) of Formula III at the 5'-end of the sense strand, the antisense strand, or both strands. In anther non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end of the sense strand, the antisense strand, or both strands.

In another embodiment, a siNA molecule of the invention comprises a nucleotide having Formula II or III, wherein the nucleotide having Formula II or III is in an inverted configuration. For example, the nucleotide having Formula II or III is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a 5'-terminal phosphate group having Formula IV:



wherein each X and Y is independently O, S, N, alkyl, substituted alkyl, or alkylhalo; wherein each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, or alkylhalo; and wherein W, X, Y and Z are not all O.

In one embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand, for example, a strand complementary to a target RNA, wherein the siNA molecule comprises an all RNA siNA molecule. In another embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand wherein the siNA molecule also comprises about 1 to about 3 (*e.g.*, about 1, 2, or 3) nucleotide 3'-terminal nucleotide overhangs having about 1 to about 4 (*e.g.*, about 1, 2, 3, or 4) deoxyribonucleotides on the 3'-end of one or both strands. In another embodiment, a 5'-terminal phosphate group having Formula IV is present on the target-complementary strand of a siNA molecule of the invention, for example a siNA molecule having chemical modifications having any of Formulae I-VII.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more phosphorothioate internucleotide linkages. For example, in a non-limiting example, the invention features a chemically-modified short interfering nucleic acid (siNA) having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in one siNA strand. In yet another embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) individually having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in both siNA strands. The phosphorothioate internucleotide linkages can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more phosphorothioate internucleotide linkages at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) consecutive phosphorothioate internucleotide linkages at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine phosphorothioate internucleotide linkages in the sense

strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a siNA molecule, wherein the sense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or about one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the sense strand comprises about 1 to about 5, specifically about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7,

8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5 or more, for example about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a siNA molecule, wherein the antisense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or about one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3' and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about

1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5, for example about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule having about 1 to about 5, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages in each strand of the siNA molecule.

In another embodiment, the invention features a siNA molecule comprising 2'-5' internucleotide linkages. The 2'-5' internucleotide linkage(s) can be at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of one or both siNA sequence strands. In addition, the 2'-5' internucleotide linkage(s) can be present at various other positions within one or both siNA sequence strands, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a pyrimidine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a purine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage.

In another embodiment, a chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified, wherein each strand is about 18 to about 27 (*e.g.*, about 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27) nucleotides in length, wherein the duplex has about 18 to about 23 (*e.g.*, about 18, 19,

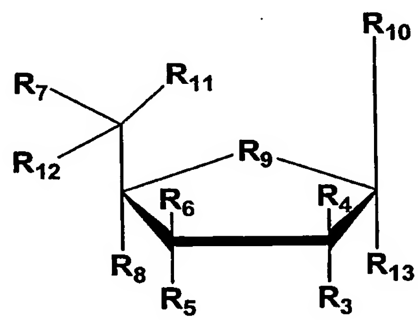
20, 21, 22, or 23) base pairs, and wherein the chemical modification comprises a structure having any of Formulae I-VII. For example, an exemplary chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein each strand consists of about 21 nucleotides, each having a 2-nucleotide 3'-terminal nucleotide overhang, and wherein the duplex has about 19 base pairs. In another embodiment, a siNA molecule of the invention comprises a single stranded hairpin structure, wherein the siNA is about 36 to about 70 (*e.g.*, about 36, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 18 to about 23 (*e.g.*, about 18, 19, 20, 21, 22, or 23) base pairs, and wherein the siNA can include a chemical modification comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 42 to about 50 (*e.g.*, about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having about 19 base pairs and a 2-nucleotide 3'-terminal nucleotide overhang. In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. For example, a linear hairpin siNA molecule of the invention is designed such that degradation of the loop portion of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

In another embodiment, a siNA molecule of the invention comprises a circular nucleic acid molecule, wherein the siNA is about 38 to about 70 (*e.g.*, about 38, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 18 to about 23 (*e.g.*, about 18, 19, 20, 21, 22, or 23) base pairs, and wherein the siNA can include a chemical modification, which comprises a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a circular oligonucleotide having about 42 to about 50 (*e.g.*, about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any

of Formulae I-VII or any combination thereof, wherein the circular oligonucleotide forms a dumbbell shaped structure having about 19 base pairs and 2 loops.

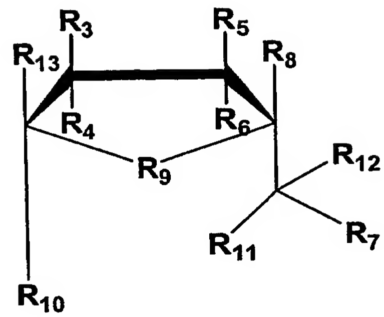
In another embodiment, a circular siNA molecule of the invention contains two loop motifs, wherein one or both loop portions of the siNA molecule is biodegradable. For example, a circular siNA molecule of the invention is designed such that degradation of the loop portions of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

In one embodiment, a siNA molecule of the invention comprises at least one (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) abasic moiety, for example a compound having Formula V:



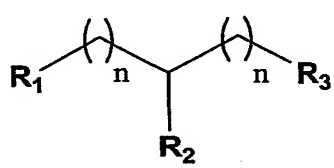
wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I; R9 is O, S, CH2, S=O, CHF, or CF2.

In one embodiment, a siNA molecule of the invention comprises at least one (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) inverted abasic moiety, for example a compound having Formula VI:



wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I; R9 is O, S, CH2, S=O, CHF, or CF2, and either R2, R3, R8 or R13 serve as points of attachment to the siNA molecule of the invention.

In another embodiment, a siNA molecule of the invention comprises at least one (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) substituted polyalkyl moieties, for example a compound having Formula VII:



wherein each n is independently an integer from 1 to 12, each R1, R2 and R3 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or a group having Formula I, and R1, R2 or R3 serves as points of attachment to the siNA molecule of the invention.

In another embodiment, the invention features a compound having Formula VII, wherein R1 and R2 are hydroxyl (OH) groups, n = 1, and R3 comprises O and is the point of attachment to the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both strands of a double-stranded siNA molecule of the invention or to a single-stranded siNA molecule of the invention. This modification is referred to herein as "glyceryl" (for example modification 6 in **Figure 10**).

In another embodiment, a moiety having any of Formula V, VI or VII of the invention is at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of a siNA molecule of the invention. For example, a moiety having Formula V, VI or VII can be present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense strand, the sense strand, or both antisense and sense strands of the siNA molecule. In addition, a moiety having Formula VII can be present at the 3'-end or the 5'-end of a hairpin siNA molecule as described herein.

In another embodiment, a siNA molecule of the invention comprises an abasic residue having Formula V or VI, wherein the abasic residue having Formula VI or VI is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, a siNA molecule of the invention comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) locked nucleic acid (LNA) nucleotides, for example at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In another embodiment, a siNA molecule of the invention comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) acyclic nucleotides, for example at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention, wherein the chemically-modified siNA comprises a sense region, where any (*e.g.,* one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a

plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and where any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention, wherein the chemically-modified siNA comprises a sense region, where any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and where any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention, wherein the chemically-modified siNA comprises an antisense region, where any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention, wherein the chemically-modified siNA comprises an antisense region, where any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein

all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said antisense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention, wherein the chemically-modified siNA comprises an antisense region, where any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and where any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention capable of mediating RNA interference (RNAi) against a VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system, wherein the chemically-modified siNA comprises a sense region, where one or more pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and where one or more purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), and inverted deoxy abasic modifications that are optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense region, the sense region optionally further comprising a 3'-terminal overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxyribonucleotides; and wherein the chemically-modified short interfering nucleic acid

molecule comprises an antisense region, where one or more pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein one or more purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in **Figure 10,** that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the antisense region optionally further comprising a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxynucleotides, wherein the overhang nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages. Non-limiting examples of these chemically-modified siNAs are shown in **Figures 4 and 5** and **Tables III and IV** herein.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention capable of mediating RNA interference (RNAi) against a VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system, wherein the siNA comprises a sense region, where one or more pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and where one or more purine nucleotides present in the sense region are purine ribonucleotides (e.g., wherein all purine nucleotides are purine ribonucleotides or alternately a plurality of purine nucleotides are purine ribonucleotides), and inverted deoxy abasic modifications that are optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense region, the sense region optionally further comprising a 3'-terminal overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxyribonucleotides; and wherein the siNA comprises an antisense region, where one or more pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of

pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in **Figure 10,** that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the antisense region optionally further comprising a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxynucleotides, wherein the overhang nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages. Non-limiting examples of these chemically-modified siNAs are shown in **Figures 4 and 5** and **Tables III and IV** herein.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention capable of mediating RNA interference (RNAi) against a VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system, wherein the chemically-modified siNA comprises a sense region, where one or more pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and for example where one or more purine nucleotides present in the sense region are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides (*e.g.*, wherein all purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides or alternately a plurality of purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides), and wherein inverted deoxy abasic modifications are optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense region, the sense region optionally further comprising a 3'-terminal overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxyribonucleotides; and wherein the chemically-modified short interfering nucleic acid

molecule comprises an antisense region, where one or more pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein one or more purine nucleotides present in the antisense region are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides (e.g., wherein all purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides or alternately a plurality of purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides), and a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the antisense region optionally further comprising a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxynucleotides, wherein the overhang nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages.

In another embodiment, any modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, are resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi. Non-limiting examples of nucleotides having a northern configuration include locked nucleic acid (LNA)

nucleotides (e.g., 2′-O, 4′-C-methylene-(D-ribofuranosyl) nucleotides); 2'-methoxyethoxy (MOE) nucleotides; 2'-methyl-thio-ethyl, 2'-deoxy-2'-fluoro nucleotides, 2'-deoxy-2'-chloro nucleotides, 2'-azido nucleotides, and 2'-O-methyl nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid molecule (siNA) capable of mediating RNA interference (RNAi) against a VEGF and/or VEGFr inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a conjugate covalently attached to the chemically-modified siNA molecule. In another embodiment, the conjugate is covalently attached to the chemically-modified siNA molecule via a biodegradable linker. In one embodiment, the conjugate molecule is attached at the 3'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In another embodiment, the conjugate molecule is attached at the 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In yet another embodiment, the conjugate molecule is attached both the 3'-end and 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule, or any combination thereof. In one embodiment, a conjugate molecule of the invention comprises a molecule that facilitates delivery of a chemically-modified siNA molecule into a biological system, such as a cell. In another embodiment, the conjugate molecule attached to the chemically-modified siNA molecule is a poly ethylene glycol, human serum albumin, or a ligand for a cellular receptor that can mediate cellular uptake. Examples of specific conjugate molecules contemplated by the instant invention that can be attached to chemically-modified siNA molecules are described in Vargeese *et al.,* U.S. Serial No. 10/201,394, incorporated by reference herein. The type of conjugates used and the extent of conjugation of siNA molecules of the invention can be evaluated for improved pharmacokinetic profiles, bioavailability, and/or stability of siNA constructs while at the same time maintaining the ability of the siNA to mediate RNAi activity. As such, one skilled in the art can screen siNA constructs that are modified with various conjugates to determine whether the siNA conjugate complex possesses improved properties while maintaining the ability to mediate RNAi, for example in animal models as are generally known in the art.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule of the invention, wherein the siNA further comprises a nucleotide, non-nucleotide, or mixed nucleotide/non-nucleotide linker that joins the sense region of the siNA to the antisense region of the siNA. In one embodiment, a nucleotide linker of the invention can be a linker of ≥ 2 nucleotides in length, for example 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length. In another embodiment, the nucleotide linker can be a nucleic acid aptamer. By "aptamer" or "nucleic acid aptamer" as used herein is meant a nucleic acid molecule that binds specifically to a target molecule wherein the nucleic acid molecule has sequence that comprises a sequence recognized by the target molecule in its natural setting. Alternately, an aptamer can be a nucleic acid molecule that binds to a target molecule where the target molecule does not naturally bind to a nucleic acid. The target molecule can be any molecule of interest. For example, the aptamer can be used to bind to a ligand-binding domain of a protein, thereby preventing interaction of the naturally occurring ligand with the protein. This is a non-limiting example and those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. (See, for example, Gold *et al.,* 1995, *Annu. Rev. Biochem.,* 64, 763; Brody and Gold, 2000, *J. Biotechnol.,* 74, 5; Sun, 2000, *Curr. Opin. Mol. Ther.,* 2, 100; Kusser, 2000, *J. Biotechnol.,* 74, 27; Hermann and Patel, 2000, *Science,* 287, 820; and Jayasena, 1999, *Clinical Chemistry,* 45, 1628.)

In yet another embodiment, a non-nucleotide linker of the invention comprises abasic nucleotide, polyether, polyamine, polyamide, peptide, carbohydrate, lipid, polyhydrocarbon, or other polymeric compounds (e.g. polyethylene glycols such as those having between 2 and 100 ethylene glycol units). Specific examples include those described by Seela and Kaiser, *Nucleic Acids Res.* 1990, *18*:6353 and *Nucleic Acids Res.* 1987, *15*:3113; Cload and Schepartz, *J. Am. Chem. Soc.* 1991, *113*:6324; Richardson and Schepartz, *J. Am. Chem. Soc.* 1991, *113*:5109; Ma *et al., Nucleic Acids Res.* 1993, *21*:2585 and *Biochemistry* 1993, *32*:1751; Durand *et al., Nucleic Acids Res.* 1990, *18*:6353; McCurdy *et al., Nucleosides & Nucleotides* 1991, *10*:287; Jschke *et al., Tetrahedron Lett.* 1993, *34*:301; Ono *et al., Biochemistry* 1991, *30*:9914; Arnold *et al.,* International Publication No. WO 89/02439; Usman *et al.,* International Publication No. WO 95/06731; Dudycz *et al.,* International Publication No. WO 95/11910 and Ferentz and Verdine, *J. Am. Chem. Soc.* 1991, *113*:4000,

all hereby incorporated by reference herein. A "non-nucleotide" further means any group or compound that can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound can be abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine, for example at the C1 position of the sugar.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) inside a cell or reconstituted in vitro system, wherein one or both strands of the siNA molecule that are assembled from two separate oligonucleotides do not comprise any ribonucleotides. For example, a siNA molecule can be assembled from a single oligonculeotide where the sense and antisense regions of the siNA comprise separate oligonucleotides not having any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotides. In another example, a siNA molecule can be assembled from a single oligonculeotide where the sense and antisense regions of the siNA are linked or circularized by a nucleotide or non-nucleotide linker as desrcibed herein, wherein the oligonucleotide does not have any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotide. Applicant has surprisingly found that the presense of ribonucleotides (e.g., nucleotides having a 2'-hydroxyl group) within the siNA molecule is not required or essential to support RNAi activity. As such, in one embodiment, all positions within the siNA can include chemically modified nucleotides and/or non-nucleotides such as nucleotides and or non-nucleotides having Formula I, II, III, IV, V, VI, or VII or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the siNA molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group and a 3'-terminal phosphate group (e.g., a 2',3'-cyclic phosphate). In another embodiment, the single

stranded siNA molecule of the invention comprises about 19 to about 29 nucleotides. In yet another embodiment, the single stranded siNA molecule of the invention comprises one or more chemically modified nucleotides or non-nucleotides described herein. For example, all the positions within the siNA molecule can include chemically-modified nucleotides such as nucleotides having any of Formulae I-VII, or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the siNA molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence, and wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the siNA optionally further comprising about 1 to about 4 (e.g., about 1, 2, 3, or 4) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the siNA molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence, and wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine

nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the siNA optionally further comprising about 1 to about 4 (e.g., about 1, 2, 3, or 4) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the siNA molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence, and wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are locked nucleic acid (LNA) nucleotides (e.g., wherein all purine nucleotides are LNA nucleotides or alternately a plurality of purine nucleotides are LNA nucleotides), and a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the siNA optionally further comprising about 1 to about 4 (e.g., about 1, 2, 3, or 4) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the siNA molecule comprises a single stranded polynucleotide having complementarity to a

target nucleic acid sequence, and wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-methoxyethyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-methoxyethyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-methoxyethyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the siNA optionally further comprising about 1 to about 4 (e.g., about 1, 2, 3, or 4) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group.

In another embodiment, any modified nucleotides present in the single stranded siNA molecules of the invention comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the single stranded siNA molecules of the invention are preferably resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi.

In one embodiment, the invention features a method for modulating the expression of a VEGF and/or VEGFr gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the cell.

In one embodiment, the invention features a method for modulating the expression of a VEGF and/or VEGFr gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr gene and wherein the sense strand sequence of the siNA comprises a sequence identical to the sequence of the target RNA; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one VEGF and/or VEGFr gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr genes; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one VEGF and/or VEGFr gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr gene and wherein the sense strand sequence of the siNA comprises a sequence identical to the sequence of the target RNA; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in the cell.

In one embodiment, siNA molecules of the invention are used as reagents in ex vivo applications. For example, siNA reagents are intoduced into tissue or cells that are transplanted into a subject for therapeutic effect. The cells and/or tissue can be derived from an organism or subject that later receives the explant, or can be derived from another organism or subject prior to transplantation. The siNA molecules can be used to modulate the expression of one or more genes in the cells or tissue, such that the cells or tissue obtain a desired phenotype or are able to perform a function when transplanted in vivo. In one embodiment, certain target cells from a patient are extracted. These extracted cells are contacted with siNAs targeteing a specific nucleotide sequence within the cells under

conditions suitable for uptake of the siNAs by these cells (e.g. using delivery reagents such as cationic lipids, liposomes and the like or using techniques such as electroporation to facilitate the delivery of siNAs into cells). The cells are then reintroduced back into the same patient or other patients. In one embodiment, the invention features a method of modulating the expression of a VEGF and/or VEGFr gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr gene; and (b) introducing the siNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in that organism.

In one embodiment, the invention features a method of modulating the expression of a VEGF and/or VEGFr gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr gene and wherein the sense strand sequence of the siNA comprises a sequence identical to the sequence of the target RNA; and (b) introducing the siNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in that organism.

In another embodiment, the invention features a method of modulating the expression of more than one VEGF and/or VEGFr gene in a tissue explant comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr genes; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the VEGF

and/or VEGFr genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in that organism.

In one embodiment, the invention features a method of modulating the expression of a VEGF and/or VEGFr gene in an organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr gene; and (b) introducing the siNA molecule into the organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the organism.

In another embodiment, the invention features a method of modulating the expression of more than one VEGF and/or VEGFr gene in an organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the VEGF and/or VEGFr genes; and (b) introducing the siNA molecules into the organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in the organism.

In one embodiment, the invention features a method for modulating the expression of a VEGF and/or VEGFr gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the VEGF and/or VEGFr gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one VEGF and/or VEGFr gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the VEGF and/or VEGFr gene; and (b) contacting the siNA molecule with a cell in vitro or in vivo under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in the cell.

In one embodiment, the invention features a method of modulating the expression of a VEGF and/or VEGFr gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the VEGF and/or VEGFr gene; and (b) contacting the siNA molecule with a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in that organism.

In another embodiment, the invention features a method of modulating the expression of more than one VEGF and/or VEGFr gene in a tissue explant comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the VEGF and/or VEGFr gene; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in that organism.

In one embodiment, the invention features a method of modulating the expression of a VEGF and/or VEGFr gene in an organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the VEGF and/or VEGFr gene; and (b) introducing the siNA molecule into the organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the organism.

In another embodiment, the invention features a method of modulating the expression of more than one VEGF and/or VEGFr gene in an organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA

comprises a single stranded sequence having complementarity to RNA of the VEGF and/or VEGFr gene; and (b) introducing the siNA molecules into the organism under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in the organism.

In one embodiment, the invention features a method of modulating the expression of a VEGF and/or VEGFr gene in an organism comprising contacting the organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the VEGF and/or VEGFr gene in the organism.

In another embodiment, the invention features a method of modulating the expression of more than one VEGF and/or VEGFr gene in an organism comprising contacting the organism with one or more siNA molecules of the invention under conditions suitable to modulate the expression of the VEGF and/or VEGFr genes in the organism.

The siNA molecules of the invention can be designed to inhibit target (VEGF and/or VEGFr) gene expression through RNAi targeting of a variety of RNA molecules. In one embodiment, the siNA molecules of the invention are used to target various RNAs corresponding to a target gene. Non-limiting examples of such RNAs include messenger RNA (mRNA), alternate RNA splice variants of target gene(s), post-transcriptionally modified RNA of target gene(s), pre-mRNA of target gene(s), and/or RNA templates. If alternate splicing produces a family of transcripts that are distinguished by usage of appropriate exons, the instant invention can be used to inhibit gene expression through the appropriate exons to specifically inhibit or to distinguish among the functions of gene family members. For example, a protein that contains an alternatively spliced transmembrane domain can be expressed in both membrane bound and secreted forms. Use of the invention to target the exon containing the transmembrane domain can be used to determine the functional consequences of pharmaceutical targeting of membrane bound as opposed to the secreted form of the protein. Non-limiting examples of applications of the invention relating to targeting these RNA molecules include therapeutic pharmaceutical applications, pharmaceutical discovery applications, molecular diagnostic and gene function applications, and gene mapping, for example using single nucleotide polymorphism mapping with siNA

molecules of the invention. Such applications can be implemented using known gene sequences or from partial sequences available from an expressed sequence tag (EST).

In another embodiment, the siNA molecules of the invention are used to target conserved sequences corresponding to a gene family or gene families such as VEGF and/or VEGFr family genes. As such, siNA molecules targeting multiple VEGF and/or VEGFr targets can provide increased therapeutic effect. In addition, siNA can be used to characterize pathways of gene function in a variety of applications. For example, the present invention can be used to inhibit the activity of target gene(s) in a pathway to determine the function of uncharacterized gene(s) in gene function analysis, mRNA function analysis, or translational analysis. The invention can be used to determine potential target gene pathways involved in various diseases and conditions toward pharmaceutical development. The invention can be used to understand pathways of gene expression involved in, for example, the progression and/or maintenance of cancer.

In one embodiment, siNA molecule(s) and/or methods of the invention are used to inhibit the expression of gene(s) that encode RNA referred to by Genbank Accession, for example VEGF and/or VEGFr genes encoding RNA sequence(s) referred to herein by Genbank Accession number, for example, Genbank Accession Nos. shown in **Table I**.

In one embodiment, the invention features a method comprising: (a) generating a library of siNA constructs having a predetermined complexity; and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target RNA sequence. In another embodiment, the siNA molecules of (a) have strands of a fixed length, for example, about 23 nucleotides in length. In yet another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 19 to about 25 (*e.g.*, about 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNAse protection assays, to determine the most suitable target site(s) within the target RNA

sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

In one embodiment, the invention features a method comprising: (a) generating a randomized library of siNA constructs having a predetermined complexity, such as of 4^N, where N represents the number of base paired nucleotides in each of the siNA construct strands (*eg.* for a siNA construct having 21 nucleotide sense and antisense strands with 19 base pairs, the complexity would be 4^{19}); and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target VEGF and/or VEGFr RNA sequence. In another embodiment, the siNA molecules of (a) have strands of a fixed length, for example about 23 nucleotides in length. In yet another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 19 to about 25 (*e.g.*, about 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described in Example 7 herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of VEGF and/or VEGFr RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNAse protection assays, to determine the most suitable target site(s) within the target VEGF and/or VEGFr RNA sequence. The target VEGF and/or VEGFr RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

In another embodiment, the invention features a method comprising: (a) analyzing the sequence of a RNA target encoded by a target gene; (b) synthesizing one or more sets of siNA molecules having sequence complementary to one or more regions of the RNA of (a); and (c) assaying the siNA molecules of (b) under conditions suitable to determine RNAi targets within the target RNA sequence. In one embodiment, the siNA molecules of (b) have strands of a fixed length, for example about 23 nucleotides in length. In another embodiment, the siNA molecules of (b) are of differing length, for example having strands of about 19 to about 25 (*e.g.*, about 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described

herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. Fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNAse protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by expression in *in vivo* systems.

By "target site" is meant a sequence within a target RNA that is "targeted" for cleavage mediated by a siNA construct which contains sequences within its antisense region that are complementary to the target sequence.

By "detectable level of cleavage" is meant cleavage of target RNA (and formation of cleaved product RNAs) to an extent sufficient to discern cleavage products above the background of RNAs produced by random degradation of the target RNA. Production of cleavage products from 1-5% of the target RNA is sufficient to detect above the background for most methods of detection.

In one embodiment, the invention features a composition comprising a siNA molecule of the invention, which can be chemically-modified, in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a pharmaceutical composition comprising siNA molecules of the invention, which can be chemically-modified, targeting one or more genes in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a method for treating or preventing a disease or condition in a subject, comprising administering to the subject a composition of the invention under conditions suitable for the treatment or prevention of the disease or condition in the subject, alone or in conjunction with one or more other therapeutic compounds. In yet another embodiment, the invention features a method for reducing or preventing tissue rejection in a subject comprising administering to the subject a composition of the invention under conditions suitable for the reduction or prevention of tissue rejection in the subject.

In another embodiment, the invention features a method for validating a VEGF and/or VEGFr gene target, comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a VEGF and/or VEGFr target gene; (b) introducing the siNA molecule into a cell, tissue, or organism under conditions suitable for modulating expression of the VEGF and/or VEGFr target gene in the cell, tissue, or organism; and (c) determining the function of the gene by assaying for any phenotypic change in the cell, tissue, or organism.

In another embodiment, the invention features a method for validating a VEGF and/or VEGFr target comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a VEGF and/or VEGFr target gene; (b) introducing the siNA molecule into a biological system under conditions suitable for modulating expression of the VEGF and/or VEGFr target gene in the biological system; and (c) determining the function of the gene by assaying for any phenotypic change in the biological system.

By "biological system" is meant, material, in a purified or unpurified form, from biological sources, including but not limited to human, animal, plant, insect, bacterial, viral or other sources, wherein the system comprises the components required for RNAi acitivity. The term "biological system" includes, for example, a cell, tissue, or organism, or extract thereof. The term biological system also includes reconstituted RNAi systems that can be used in an *in vitro* setting.

By "phenotypic change" is meant any detectable change to a cell that occurs in response to contact or treatment with a nucleic acid molecule of the invention (e.g., siNA). Such detectable changes include, but are not limited to, changes in shape, size, proliferation, motility, protein expression or RNA expression or other physical or chemical changes as can be assayed by methods known in the art. The detectable change can also include expression of reporter genes/molecules such as Green Florescent Protein (GFP) or various tags that are used to identify an expressed protein or any other cellular component that can be assayed.

In one embodiment, the invention features a kit containing a siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of a VEGF and/or VEGFr target gene in a cell, tissue, or organism. In another embodiment, the invention features a kit containing more than one siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of more than one VEGF and/or VEGFr target gene in a cell, tissue, or organism.

In one embodiment, the invention features a cell containing one or more siNA molecules of the invention, which can be chemically-modified. In another embodiment, the cell containing a siNA molecule of the invention is a mammalian cell. In yet another embodiment, the cell containing a siNA molecule of the invention is a human cell.

In one embodiment, the synthesis of a siNA molecule of the invention, which can be chemically-modified, comprises: (a) synthesis of two complementary strands of the siNA molecule; (b) annealing the two complementary strands together under conditions suitable to obtain a double-stranded siNA molecule. In another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase oligonucleotide synthesis. In yet another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase tandem oligonucleotide synthesis.

In one embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing a first oligonucleotide sequence strand of the siNA molecule, wherein the first oligonucleotide sequence strand comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of the second oligonucleotide sequence strand of the siNA; (b) synthesizing the second oligonucleotide sequence strand of siNA on the scaffold of the first oligonucleotide sequence strand, wherein the second oligonucleotide sequence strand further comprises a chemical moiety than can be used to purify the siNA duplex; (c) cleaving the linker molecule of (a) under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex; and (d) purifying the siNA duplex utilizing the chemical moiety of the second oligonucleotide sequence strand. In one embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example under hydrolysis conditions

using an alkylamine base such as methylamine. In one embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place concomitantly. In another embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group, which can be employed in a trityl-on synthesis strategy as described herein. In yet another embodiment, the chemical moiety, such as a dimethoxytrityl group, is removed during purification, for example, using acidic conditions.

In a further embodiment, the method for siNA synthesis is a solution phase synthesis or hybrid phase synthesis wherein both strands of the siNA duplex are synthesized in tandem using a cleavable linker attached to the first sequence which acts a scaffold for synthesis of the second sequence. Cleavage of the linker under conditions suitable for hybridization of the separate siNA sequence strands results in formation of the double-stranded siNA molecule.

In another embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing one oligonucleotide sequence strand of the siNA molecule, wherein the sequence comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of another oligonucleotide sequence; (b) synthesizing a second oligonucleotide sequence having complementarity to the first sequence strand on the scaffold of (a), wherein the second sequence comprises the other strand of the double-stranded siNA molecule and wherein the second sequence further comprises a chemical moiety than can be used to isolate the attached oligonucleotide sequence; (c) purifying the product of (b) utilizing the chemical moiety of the second oligonucleotide sequence strand under conditions suitable for isolating the full-length sequence comprising both siNA oligonucleotide strands connected by the cleavable linker and under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex. In one

embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example under hydrolysis conditions. In another embodiment, cleavage of the linker molecule in (c) above takes place after deprotection of the oligonucleotide. In another embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity or differing reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place either concomitantly or sequentially. In one embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group.

In another embodiment, the invention features a method for making a double-stranded siNA molecule in a single synthetic process comprising: (a) synthesizing an oligonucleotide having a first and a second sequence, wherein the first sequence is complementary to the second sequence, and the first oligonucleotide sequence is linked to the second sequence via a cleavable linker, and wherein a terminal 5'-protecting group, for example, a 5'-O-dimethoxytrityl group (5'-O-DMT) remains on the oligonucleotide having the second sequence; (b) deprotecting the oligonucleotide whereby the deprotection results in the cleavage of the linker joining the two oligonucleotide sequences; and (c) purifying the product of (b) under conditions suitable for isolating the double-stranded siNA molecule, for example using a trityl-on synthesis strategy as described herein.

In another embodiment, the method of synthesis of siNA molecules of the invention comprises the teachings of Scaringe *et al.*, US Patent Nos. 5,889,136; 6,008,400; and 6,111,086, incorporated by reference herein in their entirety.

In one embodiment, the invention features siNA constructs that mediate RNAi against a VEGF and/or VEGFr, wherein the siNA construct comprises one or more chemical modifications, for example, one or more chemical modifications having any of Formulae I-VII or any combination thereof that increases the nuclease resistance of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules with increased nuclease resistance comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased nuclease resistance.

In one embodiment, the invention features siNA constructs that mediate RNAi against a VEGF and/or VEGFr, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the sense and antisense strands of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the sense and antisense strands of the siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the sense and antisense strands of the siNA molecule.

In one embodiment, the invention features siNA constructs that mediate RNAi against a VEGF and/or VEGFr, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target RNA sequence within a cell.

In one embodiment, the invention features siNA constructs that mediate RNAi against a VEGF and/or VEGFr, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target DNA sequence within a cell.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having

increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence.

In one embodiment, the invention features siNA constructs that mediate RNAi against a VEGF and/or VEGFr, wherein the siNA construct comprises one or more chemical modifications described herein that modulate the polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA construct.

In another embodiment, the invention features a method for generating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to a chemically-modified siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA molecule.

In one embodiment, the invention features chemically-modified siNA constructs that mediate RNAi against a VEGF and/or VEGFr in a cell, wherein the chemical modifications do not significantly effect the interaction of siNA with a target RNA molecule, DNA molecule and/or proteins or other factors that are essential for RNAi in a manner that would decrease the efficacy of RNAi mediated by such siNA constructs.

In another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against VEGF and/or VEGFr comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity.

In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against a VEGF and/or VEGFr target RNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target RNA.

In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against a VEGF and/or VEGFr target DNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target DNA.

In one embodiment, the invention features siNA constructs that mediate RNAi against a VEGF and/or VEGFr, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the cellular uptake of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules against VEGF and/or VEGFr with improved cellular uptake comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved cellular uptake.

In one embodiment, the invention features siNA constructs that mediate RNAi against a VEGF and/or VEGFr, wherein the siNA construct comprises one or more chemical modifications described herein that increases the bioavailability of the siNA construct, for

example, by attaching polymeric conjugates such as polyethyleneglycol or equivalent conjugates that improve the pharmacokinetics of the siNA construct, or by attaching conjugates that target specific tissue types or cell types *in vivo*. Non-limiting examples of such conjugates are described in Vargeese *et al.*, U.S. Serial No. 10/201,394 incorporated by reference herein.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability, comprising (a) introducing a conjugate into the structure of a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such conjugates can include ligands for cellular receptors, such as peptides derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code sequences; antibodies; nucleic acid aptamers; vitamins and other co-factors, such as folate and N-acetylgalactosamine; polymers, such as polyethyleneglycol (PEG); phospholipids; polyamines, such as spermine or spermidine; and others.

In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing an excipient formulation to a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such excipients include polymers such as cyclodextrins, lipids, cationic lipids, polyamines, phospholipids, and others.

In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing nucleotides having any of Formulae I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability.

In another embodiment, polyethylene glycol (PEG) can be covalently attached to siNA compounds of the present invention. The attached PEG can be any molecular weight, preferably from about 2,000 to about 50,000 daltons (Da).

The present invention can be used alone or as a component of a kit having at least one of the reagents necessary to carry out the *in vitro* or *in vivo* introduction of RNA to test samples and/or subjects. For example, preferred components of the kit include a siNA molecule of the invention and a vehicle that promotes introduction of the siNA into cells of interest as described herein (e.g., using lipids and other methods of transfection known in the art, see for example Beigelman *et al,* US 6,395,713). The kit can be used for target validation, such as in determining gene function and/or activity, or in drug optimization, and in drug discovery (see for example Usman et al., USSN 60/402,996). Such a kit can also include instructions to allow a user of the kit to practice the invention.

The term "short interfering nucleic acid", "siNA", "short interfering RNA", "siRNA", "short interfering nucleic acid molecule", "short interfering oligonucleotide molecule", or "chemically-modified short interfering nucleic acid molecule" as used herein refers to any nucleic acid molecule capable of inhibiting or down regulating gene expression, for example by mediating RNA interference "RNAi" or gene silencing in a sequence-specific manner; see for example Bass, 2001, *Nature*, 411, 428-429; Elbashir *et al.*, 2001, *Nature*, 411, 494-498; and Kreutzer *et al.*, International PCT Publication No. WO 00/44895; Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck *et al.*, International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li *et al.*, International PCT Publication No. WO 00/44914; Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237; Hutvagner and Zamore, 2002, *Science*, 297, 2056-60; McManus *et al.*, 2002, *RNA*, 8, 842-850; Reinhart *et al.*, 2002, *Gene & Dev.*, 16, 1616-1626; and Reinhart & Bartel, 2002, *Science*, 297, 1831). Non limiting examples of siNA molecules of the invention are shown in **Figures 4-6,** and **Tables II, III, and IV** herein. For example the siNA can be a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic

acid sequence or a portion thereof. The siNA can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (i.e. each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double stranded structure, for example wherein the double stranded region is about 19 base pairs); the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. Alternatively, the siNA is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the siNA are linked by means of a nucleic acid based or non-nucleic acid-based linker(s). The siNA can be a polynucleotide with a hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide can be processed either *in vivo* or *in vitro* to generate an active siNA molecule capable of mediating RNAi. The siNA can also comprise a single stranded polynucleotide having nucleotide sequence complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof (for example, where such siNA molecule does not require the presence within the siNA molecule of nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof), wherein the single stranded polynucleotide can further comprise a terminal phosphate group, such as a 5'-phosphate (see for example Martinez *et al.*, 2002, *Cell.*, 110, 563-574 and Schwarz *et al.*, 2002, *Molecular Cell*, 10, 537-568), or 5',3'-diphosphate. In certain embodiment, the siNA molecule of the invention comprises separate sense and antisense

sequences or regions, wherein the sense and antisense regions are covalently linked by nucleotide or non-nucleotide linkers molecules as is known in the art, or are alternately non-covalently linked by ionic interactions, hydrogen bonding, van der waals interactions, hydrophobic intercations, and/or stacking interactions. In certain embodiments, the siNA molecules of the invention comprise nucleotide sequence that is complementary to nucleotide sequence of a target gene. In another embodiment, the siNA molecule of the invention interacts with nucleotide sequence of a target gene in a manner that causes inhibition of expression of the target gene. As used herein, siNA molecules need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides. In certain embodiments, the short interfering nucleic acid molecules of the invention lack 2'-hydroxy (2'-OH) containing nucleotides. Applicant describes in certain embodiments short interfering nucleic acids that do not require the presence of nucleotides having a 2'-hydroxy group for mediating RNAi and as such, short interfering nucleic acid molecules of the invention optionally do not include any ribonucleotides (e.g., nucleotides having a 2'-OH group). Such siNA molecules that do not require the presence of ribonucleotides within the siNA molecule to support RNAi can however have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. Optionally, siNA molecules can comprise ribonucleotides at about 5, 10, 20, 30, 40, or 50% of the nucleotide positions. The modified short interfering nucleic acid molecules of the invention can also be referred to as short interfering modified oligonucleotides "siMON." As used herein, the term siNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically-modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, or epigenetics. For example, siNA molecules of the invention can be used to epigenetically silence genes at both the post-transcriptional level or the pre-transcriptional level. In a non-limiting example, epigenetic regulation of gene expression by

siNA molecules of the invention can result from siNA mediated modification of chromatin structure to alter gene expression (see, for example, Allshire, 2002, *Science,* 297, 1818-1819; Volpe *et al.,* 2002, *Science,* 297, 1833-1837; Jenuwein, 2002, *Science,* 297, 2215-2218; and Hall *et al.,* 2002, *Science,* 297, 2232-2237).

By "modulate" is meant that the expression of the gene, or level of RNA molecule or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits is up regulated or down regulated, such that expression, level, or activity is greater than or less than that observed in the absence of the modulator. For example, the term "modulate" can mean "inhibit," but the use of the word "modulate" is not limited to this definition.

By "inhibit", "down-regulate", or "reduce", it is meant that the expression of the gene, or level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits, is reduced below that observed in the absence of the nucleic acid molecules (e.g., siNA) of the invention. In one embodiment, inhibition, down-regulation or reduction with an siNA molecule is below that level observed in the presence of an inactive or attenuated molecule. In another embodiment, inhibition, down-regulation, or reduction with siNA molecules is below that level observed in the presence of, for example, an siNA molecule with scrambled sequence or with mismatches. In another embodiment, inhibition, down-regulation, or reduction of gene expression with a nucleic acid molecule of the instant invention is greater in the presence of the nucleic acid molecule than in its absence.

By "gene" or "target gene" is meant, a nucleic acid that encodes an RNA, for example, nucleic acid sequences including, but not limited to, structural genes encoding a polypeptide. The target gene can be a gene derived from a cell, an endogenous gene, a transgene, or exogenous genes such as genes of a pathogen, for example a virus, which is present in the cell after infection thereof. The cell containing the target gene can be derived from or contained in any organism, for example a plant, animal, protozoan, virus, bacterium, or fungus. Non-limiting examples of plants include monocots, dicots, or gymnosperms. Non-

limiting examples of animals include vertebrates or invertebrates. Non-limiting examples of fungi include molds or yeasts.

By "VEGF" as used herein is meant, any vascular endothelial growth factor (e.g., VEGF, VEGF-A, VEGF-B, VEGF-C, VEGF-D) protein, peptide, or polypeptide having vascular endothelial growth factor activity, such as encoded by VEGF Genbank Accession Nos. shown in **Table I.** The term VEGF also refers to nucleic acid sequences encloding any vascular endothelial growth factor protein, peptide, or polypeptide having vascular endothelial growth factor activity.

By "VEGF-B" is meant, protein, peptide, or polypeptide receptor or a derivative thereof, such as encoded by Genbank Accession No. NM_003377, having vascular endothelial growth factor type B activity. The term VEGF-B also refers to nucleic acid sequences encloding any VEGF-B protein, peptide, or polypeptide having VEGF-B activity.

By "VEGF-C" is meant, protein, peptide, or polypeptide receptor or a derivative thereof, such as encoded by Genbank Accession No. NM_005429, having vascular endothelial growth factor type C activity. The term VEGF-C also refers to nucleic acid sequences encloding any VEGF-C protein, peptide, or polypeptide having VEGF-C activity.

By "VEGF-D" is meant, protein, peptide, or polypeptide receptor or a derivative thereof, such as encoded by Genbank Accession No. NM_004469, having vascular endothelial growth factor type D activity. The term VEGF-D also refers to nucleic acid sequences encloding any VEGF-D protein, peptide, or polypeptide having VEGF-D activity.

By "VEGFr" as used herein is meant, any vascular endothelial growth factor receptor protein, peptide, or polypeptide (e.g., VEGFr1, VEGFr2, or VEGFr3, including both membrane bound and/or soluble forms thereof) having vascular endothelial growth factor receptor activity, such as encoded by VEGFr Genbank Accession Nos. shown in **Table I.** The term VEGFr also refers to nucleic acid sequences encloding any vascular endothelial growth factor receptor protein, peptide, or polypeptide having vascular endothelial growth factor receptor activity.

By "VEGFr1" is meant, protein, peptide, or polypeptide receptor or a derivative thereof, such as encoded by Genbank Accession No. NM_002019, having vascular endothelial growth factor receptor type 1 (*flt*) activity, for example, having the ability to bind a vascular endothelial growth factor. The term VEGF1 also refers to nucleic acid sequences enclding any VEGFr1 protein, peptide, or polypeptide having VEGFr1 activity.

By "VEGFr2" is meant, protein, peptide, or polypeptide receptor or a derivative thereof, such as encoded by Genbank Accession No. NM_002253, having vascular endothelial growth factor receptor type 2 (*kdr*) activity, for example, having the ability to bind a vascular endothelial growth factor. The term VEGF2 also refers to nucleic acid sequences enclding any VEGFr2 protein, peptide, or polypeptide having VEGFr2 activity.

By "VEGFr3" is meant, protein, peptide, or polypeptide receptor or a derivative thereof, such as encoded by Genbank Accession No. NM_002020 having vascular endothelial growth factor receptor type 3 (*kdr*) activity, for example, having the ability to bind a vascular endothelial growth factor. The term VEGF3 also refers to nucleic acid sequences enclding any VEGFr3 protein, peptide, or polypeptide having VEGFr3 activity.

By "highly conserved sequence region" is meant, a nucleotide sequence of one or more regions in a target gene does not vary significantly from one generation to the other or from one biological system to the other.

By "sense region" is meant a nucleotide sequence of a siNA molecule having complementarity to an antisense region of the siNA molecule. In addition, the sense region of a siNA molecule can comprise a nucleic acid sequence having homology with a target nucleic acid sequence.

By "antisense region" is meant a nucleotide sequence of a siNA molecule having complementarity to a target nucleic acid sequence. In addition, the antisense region of a siNA molecule can optionally comprise a nucleic acid sequence having complementarity to a sense region of the siNA molecule.

By "target nucleic acid" is meant any nucleic acid sequence whose expression or activity is to be modulated. The target nucleic acid can be DNA or RNA.

By "complementarity" is meant that a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., RNAi activity. Determination of binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner *et al.,* 1987, *CSH Symp. Quant. Biol.* LII pp.123-133; Frier *et al.,* 1986, *Proc. Nat. Acad. Sci.* USA 83:9373-9377; Turner *et al.,* 1987, *J. Am. Chem. Soc.* 109:3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence.

The siRNA molecules of the invention represent a novel therapeutic approach to treat a variety of pathologic indications or other conditions, such as tumor angiogenesis and cancer, including but not limited to breast cancer, lung cancer (including non-small cell lung carcinoma), prostate cancer, colorectal cancer, brain cancer, esophageal cancer, bladder cancer, pancreatic cancer, cervical cancer, head and neck cancer, skin cancers, nasopharyngeal carcinoma, liposarcoma, epithelial carcinoma, renal cell carcinoma, gallbladder adeno carcinoma, parotid adenocarcinoma, ovarian cancer, melanoma, lymphoma, glioma, endometrial sarcoma, multidrug resistant cancers, diabetic retinopathy, macular degeneration, neovascular glaucoma, myopic degeneration, arthritis, psoriasis, endometriosis, female reproduction, verruca vulgaris, angiofibroma of tuberous sclerosis, pot-wine stains, Sturge Weber syndrome, Kippel-Trenaunay-Weber syndrome, Osler-Weber-Rendu syndrome, renal disease such as Autosomal dominant polycystic kidney disease (ADPKD), and any other diseases or conditions that are related to or will respond to the levels of VEGF, VEGFr1, VEGFr2 and/or VEGFr3 in a cell or tissue, alone or in

combination with other therapies. The reduction of VEGF, VEGFr1, VEGFr2 and/or VEGFr3 expression (specifically VEGF, VEGFr1, VEGFr2 and/or VEGFr3 gene RNA levels) and thus reduction in the level of the respective protein relieves, to some extent, the symptoms of the disease or condition.ue

In one embodiment of the present invention, each sequence of a siNA molecule of the invention is independently about 18 to about 24 nucleotides in length, in specific embodiments about 18, 19, 20, 21, 22, 23, or 24 nucleotides in length. In another embodiment, the siNA duplexes of the invention independently comprise about 17 to about 23 base pairs (*e.g.*, about 17, 18, 19, 20, 21, 22 or 23). In yet another embodiment, siNA molecules of the invention comprising hairpin or circular structures are about 35 to about 55 (*e.g.*, about 35, 40, 45, 50 or 55) nucleotides in length, or about 38 to about 44 (*e.g.*, 38, 39, 40, 41, 42, 43 or 44) nucleotides in length and comprising about 16 to about 22 (*e.g.*, about 16, 17, 18, 19, 20, 21 or 22) base pairs. Exemplary siNA molecules of the invention are shown in **Table II**. Exemplary synthetic siNA molecules of the invention are shown in **Tables III and IV** and/or **Figures 4-5**.

As used herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human. The cell can be present in an organism, e.g., birds, plants and mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell). The cell can be of somatic or germ line origin, totipotent or pluripotent, dividing or non-dividing. The cell can also be derived from or can comprise a gamete or embryo, a stem cell, or a fully differentiated cell.

The siNA molecules of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or tissues. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through injection, infusion pump or stent, with or without their incorporation in biopolymers. In particular embodiments, the nucleic acid molecules of the invention comprise sequences shown in **Tables II-III** and/or **Figures 4-5**. Examples of such nucleic acid molecules consist essentially of sequences defined in these tables and figures.

Furthermore, the chemically modified constructs described in **Table IV** can be applied to any siNA sequence of the invention.

In another aspect, the invention provides mammalian cells containing one or more siNA molecules of this invention. The one or more siNA molecules can independently be targeted to the same or different sites.

By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position of a β-D-ribo-furanose moiety. The terms include double-stranded RNA, single-stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siNA or internally, for example at one or more nucleotides of the RNA. Nucleotides in the RNA molecules of the instant invention can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA.

By "subject" is meant an organism, which is a donor or recipient of explanted cells or the cells themselves. "Subject" also refers to an organism to which the nucleic acid molecules of the invention can be administered. In one embodiment, a subject is a mammal or mammalian cells. In another embodiment, a subject is a human or human cells.

The term "phosphorothioate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise a sulfur atom. Hence, the term phosphorothioate refers to both phosphorothioate and phosphorodithioate internucleotide linkages.

The term "universal base" as used herein refers to nucleotide base analogs that form base pairs with each of the natural DNA/RNA bases with little discrimination between them. Non-limiting examples of universal bases include C-phenyl, C-naphthyl and other aromatic derivatives, inosine, azole carboxamides, and nitroazole derivatives such as 3-nitropyrrole,

4-nitroindole, 5-nitroindole, and 6-nitroindole as known in the art (see for example Loakes, 2001, *Nucleic Acids Research,* 29, 2437-2447).

The term "acyclic nucleotide" as used herein refers to any nucleotide having an acyclic ribose sugar, for example where any of the ribose carbons (C1, C2, C3, C4, or C5), are independently or in combination absent from the nucleotide.

The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to treat diseases or conditions discussed herein (e.g., cancers and othe proliferative conditions). For example, to treat a particular disease or condition, the siNA molecules can be administered to a subject or can be administered to other appropriate cells evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment.

In a further embodiment, the siNA molecules can be used in combination with other known treatments to treat conditions or diseases discussed above. For example, the described molecules could be used in combination with one or more known therapeutic agents to treat a disease or condition. Non-limiting examples of other therapeutic agents that can be readily combined with a siNA molecule of the invention are enzymatic nucleic acid molecules, allosteric nucleic acid molecules, antisense, decoy, or aptamer nucleic acid molecules, antibodies such as monoclonal antibodies, small molecules, and other organic and/or inorganic compounds including metals, salts and ions.

In one embodiment, the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention, in a manner which allows expression of the siNA molecule. For example, the vector can contain sequence(s) encoding both strands of a siNA molecule comprising a duplex. The vector can also contain sequence(s) encoding a single nucleic acid molecule that is self-complementary and thus forms a siNA molecule. Non-limiting examples of such expression vectors are described in Paul *et al.,* 2002, *Nature Biotechnology,* 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology,* 19, 497; Lee *et al.,* 2002, *Nature Biotechnology,* 19, 500; and Novina *et al.,* 2002, *Nature Medicine,* advance online publication doi:10.1038/nm725.

In another embodiment, the invention features a mammalian cell, for example, a human cell, including an expression vector of the invention.

In yet another embodiment, the expression vector of the invention comprises a sequence for a siNA molecule having complementarity to a RNA molecule referred to by a Genbank Accession numbers, for example Genbank Accession Nos. shown in **Table I.**

In one embodiment, an expression vector of the invention comprises a nucleic acid sequence encoding two or more siNA molecules, which can be the same or different.

In another aspect of the invention, siNA molecules that interact with target RNA molecules and down-regulate gene encoding target RNA molecules (for example target RNA molecules referred to by Genbank Accession numbers herein) are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecules bind and down-regulate gene function or expression via RNA interference (RNAi). Delivery of siNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a non-limiting example of a scheme for the synthesis of siNA molecules. The complementary siNA sequence strands, strand 1 and strand 2, are synthesized in tandem and are connected by a cleavable linkage, such as a nucleotide succinate or abasic succinate, which can be the same or different from the cleavable linker used for solid phase synthesis on a solid support. The synthesis can be either solid phase or solution phase, in the example shown, the synthesis is a solid phase synthesis. The synthesis is performed such that a protecting group, such as a dimethoxytrityl group, remains intact on the terminal nucleotide of the tandem oligonucleotide. Upon cleavage and deprotection of the oligonucleotide, the two siNA strands spontaneously hybridize to form a siNA duplex, which allows the purification of the duplex by utilizing the properties of the terminal protecting group, for example by applying a trityl on purification method wherein only duplexes/oligonucleotides with the terminal protecting group are isolated.

Figure 2 shows a MALDI-TOV mass spectrum of a purified siNA duplex synthesized by a method of the invention. The two peaks shown correspond to the predicted mass of the separate siNA sequence strands. This result demonstrates that the siNA duplex generated from tandem synthesis can be purified as a single entity using a simple trityl-on purification methodology.

Figure 3 shows a non-limiting proposed mechanistic representation of target RNA degradation involved in RNAi. Double-stranded RNA (dsRNA), which is generated by RNA-dependent RNA polymerase (RdRP) from foreign single-stranded RNA, for example viral, transposon, or other exogenous RNA, activates the DICER enzyme that in turn generates siNA duplexes. Alternately, synthetic or expressed siNA can be introduced directly into a cell by appropriate means. An active siNA complex forms which recognizes a target RNA, resulting in degradation of the target RNA by the RISC endonuclease complex or in the synthesis of additional RNA by RNA-dependent RNA polymerase (RdRP), which can activate DICER and result in additional siNA molecules, thereby amplifying the RNAi response.

Figure 4A-F shows non-limiting examples of chemically-modified siNA constructs of the present invention. In the figure, N stands for any nucleotide (adenosine, guanosine, cytosine, uridine, or optionally thymidine, for example thymidine can be substituted in the overhanging regions designated by parenthesis (N N). Various modifications are shown for the sense and antisense strands of the siNA constructs.

Figure 4A: The sense strand comprises 21 nucleotides having four phosphorothioate 5'- and 3'-terminal internucleotide linkages, wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and four 5'-terminal phosphorothioate internucleotide linkages and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

Figure 4B: The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

Figure 4C: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

Figure 4D: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein and wherein and all purine nucleotides that may be present are 2'-deoxy nucleotides. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

Figure 4E: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the

two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

Figure 4F: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-deoxy nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand of constructs A-F comprise sequence complementary to any target nucleic acid sequence of the invention.

Figure 5A-F shows non-limiting examples of specific chemically-modified siNA sequences of the invention. **A-F** applies the chemical modifications described in **Figure 4A-F** to a VEGFr1 siNA sequence. Such chemical modifications can be applied to any sequence herein, such as any VEGF, VEGFr1, VEGFr2, or VEGFr3 sequence.

Figure 6 shows non-limiting examples of different siNA constructs of the invention. The examples shown (constructs 1, 2, and 3) have 19 representative base pairs; however, different embodiments of the invention include any number of base pairs described herein. Bracketed regions represent nucleotide overhangs, for example comprising about 1, 2, 3, or 4 nucleotides in length, preferably about 2 nucleotides. Constructs 1 and 2 can be used independently for RNAi activity. Construct 2 can comprise a polynucleotide or non-nucleotide linker, which can optionally be designed as a biodegradable linker. In one

embodiment, the loop structure shown in construct 2 can comprise a biodegradable linker that results in the formation of construct 1 *in vivo* and/or *in vitro*. In another example, construct 3 can be used to generate construct 2 under the same principle wherein a linker is used to generate the active siNA construct 2 *in vivo* and/or *in vitro*, which can optionally utilize another biodegradable linker to generate the active siNA construct 1 *in vivo* and/or *in vitro*. As such, the stability and/or activity of the siNA constructs can be modulated based on the design of the siNA construct for use *in vivo* or *in vitro* and/or *in vitro*.

Figure 7A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate siNA hairpin constructs.

Figure 7A: A DNA oligomer is synthesized with a 5'-restriction site (R1) sequence followed by a region having sequence identical (sense region of siNA) to a predetermined VEGF and/or VEGFr target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, which is followed by a loop sequence of defined sequence (X), comprising, for example, about 3 to about 10 nucleotides.

Figure 7B: The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence that will result in a siNA transcript having specificity for a VEGF and/or VEGFr target sequence and having self-complementary sense and antisense regions.

Figure 7C: The construct is heated (for example to about 95°C) to linearize the sequence, thus allowing extension of a complementary second DNA strand using a primer to the 3'-restriction sequence of the first strand. The double-stranded DNA is then inserted into an appropriate vector for expression in cells. The construct can be designed such that a 3'-terminal nucleotide overhang results from the transcription, for example by engineering restriction sites and/or utilizing a poly-U termination region as described in Paul *et al.*, 2002, *Nature Biotechnology*, 29, 505-508.

Figure 8A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate double-stranded siNA constructs.

Figure 8A: A DNA oligomer is synthesized with a 5'-restriction (R1) site sequence followed by a region having sequence identical (sense region of siNA) to a predetermined VEGF and/or VEGFr target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, and which is followed by a 3'-restriction site (R2) which is adjacent to a loop sequence of defined sequence (X).

Figure 8B: The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence.

Figure 8C: The construct is processed by restriction enzymes specific to R1 and R2 to generate a double-stranded DNA which is then inserted into an appropriate vector for expression in cells. The transcription cassette is designed such that a U6 promoter region flanks each side of the dsDNA which generates the separate sense and antisense strands of the siNA. Poly T termination sequences can be added to the constructs to generate U overhangs in the resulting transcript.

Figure 9A-E is a diagrammatic representation of a method used to determine target sites for siNA mediated RNAi within a particular target nucleic acid sequence, such as messenger RNA.

Figure 9A: A pool of siNA oligonucleotides are synthesized wherein the antisense region of the siNA constructs has complementarity to target sites across the target nucleic acid sequence, and wherein the sense region comprises sequence complementary to the antisense region of the siNA.

Figure 9B&C: **(Figure 9B)** The sequences are pooled and are inserted into vectors such that **(Figure 9C)** transfection of a vector into cells results in the expression of the siNA.

Figure 9D: Cells are sorted based on phenotypic change that is associated with modulation of the target nucleic acid sequence.

Figure 9E: The siNA is isolated from the sorted cells and is sequenced to identify efficacious target sites within the target nucleic acid sequence.

Figure 10 shows non-limiting examples of different stabilization chemistries (1-10) that can be used, for example, to stabilize the 3'-end of siNA sequences of the invention, including **(1)** [3-3']-inverted deoxyribose; **(2)** deoxyribonucleotide; **(3)** [5'-3']-3'-deoxyribonucleotide; **(4)** [5'-3']-ribonucleotide; **(5)** [5'-3']-3'-O-methyl ribonucleotide; **(6)** 3'-glyceryl; **(7)** [3'-5']-3'-deoxyribonucleotide; **(8)** [3'-3']-deoxyribonucleotide; **(9)** [5'-2']-deoxyribonucleotide; and **(10)** [5-3']-dideoxyribonucleotide. In addition to modified and unmodified backbone chemistries indicated in the figure, these chemistries can be combined with different backbone modifications as described herein, for example, backbone modifications having Formula I. In addition, the 2'-deoxy nucleotide shown 5' to the terminal modifications shown can be another modified or unmodified nucleotide or non-nucleotide described herein, for example modifications having any of Formulae I-VII or any combination thereof.

Figure 11 shows a non-limiting example of a strategy used to identify chemically modified siNA constructs of the invention that are nuclease resistance while preserving the ability to mediate RNAi activity. Chemical modifications are introduced into the siNA construct based on educated design parameters (e.g. introducing 2'-mofications, base modifications, backbone modifications, terminal cap modifications etc). The modified construct in tested in an appropriate system (e.g. human serum for nuclease resistance, shown, or an animal model for PK/delivery parameters). In parallel, the siNA construct is tested for RNAi activity, for example in a cell culture system such as a luciferase reporter assay). Lead siNA constructs are then identified which possess a particular characteristic while maintaining RNAi activity, and can be further modified and assayed once again. This same approach can be used to identify siNA-conjugate molecules with improved pharmacokinetic profiles, delivery, and RNAi activity.

Figure 12 shows a non-limiting example of siNA mediated inhibition of VEGF-induced angiogenesis using the rat corneal model of angiogenesis. siNA targeting site 2340 of VEGFr1 RNA 29695/29699 (shown as RPI No. sense strand/antisense strand) was compared to an inverted control siNA 29983/29984 (shown as RPI No. sense strand/antisense strand) at three different concentrations (1ug, 3ug, and 10ug) and compared to a VEGF control in which no siNA was administered. As shown in the Figure, siNA

constructs targeting VEGFr1 RNA can provide significant inhibition of angiogenesis in the rat corneal model.

Figure 13 shows a non-limiting example of reduction of VEGFr1 mRNA in A375 cells mediated by chemically-modified siNAs that target VEGFr1 mRNA. A549 cells were transfected with 0.25 ug/well of lipid complexed with 25 nM siNA. A screen of siNA constructs (Stabilization "Stab" chemistries are shown in **Table IV**, constructs are referred to by RPI number, see **Table III**) comprising Stab 4/5 chemistry (RPI 31190/31193), Stab 1/2 chemistry (RPI 31183/31186 and RPI 31184/31187), and unmodified RNA (RPI 30075/30076) were compared to untreated cells, matched chemistry inverted control siNA constructs, (RPI 31208/31211, RPI 31201/31204, RPI 31202/31205, and RPI 30077/30078) scrambled siNA control constructs (Scram1 and Scram2), and cells transfected with lipid alone (transfection control). All of the siNA constructs show significant reduction of VEGFr1 RNA expression.

DETAILED DESCRIPTION OF THE INVENTION

Mechanism of action of Nucleic Acid Molecules of the Invention

The discussion that follows discusses the proposed mechanism of RNA interference mediated by short interfering RNA as is presently known, and is not meant to be limiting and is not an admission of prior art. Applicant demonstrates herein that chemically-modified short interfering nucleic acids possess similar or improved capacity to mediate RNAi as do siRNA molecules and are expected to possess improved stability and activity *in vivo*; therefore, this discussion is not meant to be limiting only to siRNA and can be applied to siNA as a whole. By "improved capacity to mediate RNAi" or "improved RNAi activity" is meant to include RNAi activity measured *in vitro* and/or *in vivo* where the RNAi activity is a reflection of both the ability of the siNA to mediate RNAi and the stability of the siNAs of the invention. In this invention, the product of these activities can be increased *in vitro* and/or *in vivo* compared to an all RNA siRNA or a siNA containing a plurality of ribonucleotides. In some cases, the activity or stability of the siNA molecule can be

decreased (i.e., less than ten-fold), but the overall activity of the siNA molecule is enhanced *in vitro* and/or *in vivo*.

RNA interference refers to the process of sequence specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire *et al.*, 1998, *Nature*, 391, 806). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes which is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response though a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2', 5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as Dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Berstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from Dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes. Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex containing a siRNA, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence homologous to the siRNA. Cleavage of the target RNA takes place in the middle of the region complementary to the guide sequence of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). In addition, RNA interference can also involve small RNA

(e.g., micro-RNA or miRNA) mediated gene silencing, presumably though cellular mechanisms that regulate chromatin structure and thereby prevent transcription of target gene sequences (see for example Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237). As such, siNA molecules of the invention can be used to mediate gene silencing via interaction with RNA transcripts or alternately by interaction with particular gene sequences, wherein such interaction results in gene silencing either at the transcriptional level or post-transcriptional level.

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature*, 391, 806, were the first to observe RNAi in *C. elegans*. Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mouse embryos. Hammond *et al.*, 2000, *Nature*, 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.*, 2001, *Nature*, 411, 494, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in Drosophila embryonic lysates has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21 nucleotide siRNA duplexes are most active when containing two 2-nucleotide 3'-terminal nucleotide overhangs. Furthermore, substitution of one or both siRNA strands with 2'-deoxy or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of 3'-terminal siRNA nucleotides with deoxy nucleotides was shown to be tolerated. Mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.*, 2001, *Cell*, 107, 309); however, siRNA molecules lacking a 5'-phosphate are active when introduced exogenously, suggesting that 5'-phosphorylation of siRNA constructs may occur *in vivo*.

Synthesis of Nucleic acid Molecules

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small nucleic acid motifs ("small" refers to nucleic acid motifs no more than 100 nucleotides in length, preferably no more than 80 nucleotides in length, and most preferably no more than 50 nucleotides in length; *e.g.*, individual siNA oligonucleotide sequences or siNA sequences synthesized in tandem) are preferably used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of protein and/or RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

Oligonucleotides (*e.g.*, certain modified oligonucleotides or portions of oligonucleotides lacking ribonucleotides) are synthesized using protocols known in the art, for example as described in Caruthers *et al.*,. 1992, *Methods in Enzymology* 211, 3-19, Thompson *et al.*, International PCT Publication No. WO 99/54459, Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684, Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, Brennan *et al.*, 1998, *Biotechnol Bioeng.*, 61, 33-45, and Brennan, U.S. Pat. No. 6,001,311. All of these references are incorporated herein by reference. The synthesis of oligonucleotides makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 µmol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 sec coupling step for 2'-deoxy nucleotides or 2'-deoxy-2'-fluoro nucleotides. **Table V** outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 µmol scale can be performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 µL of 0.11 M = 6.6 µmol) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60 µL of 0.25 M = 15 µmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 22-fold excess (40 µL of 0.11 M = 4.4 µmol) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40 µL of 0.25 M = 10 µmol) can be used in each coupling cycle of deoxy residues

relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (PERSEPTIVE™). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

Deprotection of the DNA-based oligonucleotides is performed as follows: the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to –20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder.

The method of synthesis used for RNA including certain siNA molecules of the invention follows the procedure as described in Usman *et al.,* 1987, *J. Am. Chem. Soc.*, 109, 7845; Scaringe *et al.,* 1990, *Nucleic Acids Res.*, 18, 5433; and Wincott *et al.,* 1995, *Nucleic Acids Res.* 23, 2677-2684 Wincott *et al.,* 1997, *Methods Mol. Bio.*, 74, 59, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μmol scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. **Table V** outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μL of 0.11 M = 6.6

μmol) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60 μL of 0.25 M = 15 μmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 66-fold excess (120 μL of 0.11 M = 13.2 μmol) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120 μL of 0.25 M = 30 μmol) can be used in each coupling cycle of ribo residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (PERSEPTIVE™). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide0.05 M in acetonitrile) is used.

Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to –20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous TEA/HF/NMP solution (300 μL of a solution of 1.5 mL N-methylpyrrolidinone, 750 μL TEA and 1 mL TEA•3HF to provide a 1.4 M HF concentration) and heated to 65 °C. After 1.5 h, the oligomer is quenched with 1.5 M NH_4HCO_3.

Alternatively, for the one-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution

of 33% ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65 °C for 15 min. The vial is brought to rt. TEA•3HF (0.1 mL) is added and the vial is heated at 65 °C for 15 min. The sample is cooled at –20 °C and then quenched with 1.5 M NH_4HCO_3.

For purification of the trityl-on oligomers, the quenched NH_4HCO_3 solution is loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA for 13 min. The cartridge is then washed again with water, salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

The average stepwise coupling yields are typically >98% (Wincott *et al.,* 1995 *Nucleic Acids Res.* 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the example described above including but not limited to 96-well format.

Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example, by ligation (Moore *et al.,* 1992, *Science* 256, 9923; Draper *et al.,* International PCT publication No. WO 93/23569; Shabarova *et al.,* 1991, *Nucleic Acids Research* 19, 4247; Bellon *et al.,* 1997, *Nucleosides & Nucleotides,* 16, 951; Bellon *et al.,* 1997, *Bioconjugate Chem.* 8, 204), or by hybridization following synthesis and/or deprotection.

The siNA molecules of the invention can also be synthesized via a tandem synthesis methodology as described in Example 1 herein, wherein both siNA strands are synthesized as a single contiguous oligonucleotide fragment or strand separated by a cleavable linker which is subsequently cleaved to provide separate siNA fragments or strands that hybridize and permit purification of the siNA duplex. The linker can be a polynucleotide linker or a non-nucleotide linker. The tandem synthesis of siNA as described herein can be readily adapted to both multiwell/multiplate synthesis platforms such as 96 well or similarly larger multi-well platforms. The tandem synthesis of siNA as described herein can also be readily

adapted to large scale synthesis platforms employing batch reactors, synthesis columns and the like.

A siNA molecule can also be assembled from two distinct nucleic acid strands or fragments wherein one fragment includes the sense region and the second fragment includes the antisense region of the RNA molecule.

The nucleic acid molecules of the present invention can be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-fluoro, 2'-*O*-methyl, 2'-H (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman *et al.,* 1994, *Nucleic Acids Symp. Ser.* 31, 163). siNA constructs can be purified by gel electrophoresis using general methods or can be purified by high pressure liquid chromatography (HPLC; see Wincott *et al., supra,* the totality of which is hereby incorporated herein by reference) and re-suspended in water.

In another aspect of the invention, siNA molecules of the invention are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules.

<u>Optimizing Activity of the nucleic acid molecule of the invention.</u>

Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) can prevent their degradation by serum ribonucleases, which can increase their potency (see *e.g.,* Eckstein *et al.,* International Publication No. WO 92/07065; Perrault *et al.,* 1990 *Nature* 344, 565; Pieken *et al.,* 1991; *Science* 253, 314; Usman and Cedergren, 1992, *Trends in Biochem. Sci.* 17, 334; Usman *et al.,* International Publication No. WO 93/15187; and Rossi *et al.,* International Publication No. WO 91/03162; Sproat, U.S. Pat. No. 5,334,711; Gold *et al.,* U.S. Pat. No. 6,300,074; and Burgin *et al., supra;* all of which are incorporated by reference herein). All of the above references describe various chemical

modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules described herein. Modifications that enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant enhancement in their nuclease stability and efficacy. For example, oligonucleotides are modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-fluoro, 2'-*O*-methyl, 2'-O-allyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, *TIBS.* 17, 34; Usman *et al.,* 1994, *Nucleic Acids Symp. Ser.* 31, 163; Burgin *et al.,* 1996, *Biochemistry,* 35, 14090). Sugar modification of nucleic acid molecules have been extensively described in the art (see Eckstein *et al., International Publication* PCT No. WO 92/07065; Perrault *et al. Nature,* 1990, *344,* 565-568; Pieken *et al. Science,* 1991, *253,* 314-317; Usman and Cedergren, *Trends in Biochem. Sci.* , 1992, *17,* 334-339; Usman *et al. International Publication* PCT No. WO 93/15187; Sproat, *U.S. Pat.* No. 5,334,711 and Beigelman *et al.,* 1995, *J. Biol. Chem.,* 270, 25702; Beigelman *et al.,* International PCT publication No. WO 97/26270; Beigelman *et al.,* U.S. Pat. No. 5,716,824; Usman *et al.,* U.S. Pat. No. 5,627,053; Woolf *et al.,* International PCT Publication No. WO 98/13526; Thompson *et al.,* USSN 60/082,404 which was filed on April 20, 1998; Karpeisky *et al.,* 1998, *Tetrahedron Lett.,* 39, 1131; Earnshaw and Gait, 1998, *Biopolymers (Nucleic Acid Sciences),* 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.,* 67, 99-134; and Burlina *et al.,* 1997, *Bioorg. Med. Chem.,* 5, 1999-2010; all of the references are hereby incorporated in their totality by reference herein). Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into nucleic acid molecules without modulating catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications can be used as described herein to modify the siNA nucleic acid molecules of the instant invention so long as the ability of siNA to promote RNAi is cells is not significantly inhibited.

While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorodithioate, and/or 5'-methylphosphonate linkages improves stability, excessive modifications can cause some toxicity or decreased activity. Therefore, when designing nucleic acid molecules, the amount of these internucleotide linkages should be minimized. The reduction in the concentration of these linkages should lower toxicity, resulting in increased efficacy and higher specificity of these molecules.

Short interfering nucleic acid (siNA) molecules having chemical modifications that maintain or enhance activity are provided. Such a nucleic acid is also generally more resistant to nucleases than an unmodified nucleic acid. Accordingly, the *in vitro* and/or *in vivo* activity should not be significantly lowered. In cases in which modulation is the goal, therapeutic nucleic acid molecules delivered exogenously should optimally be stable within cells until translation of the target RNA has been modulated long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Improvements in the chemical synthesis of RNA and DNA (Wincott *et al.,* 1995, *Nucleic Acids Res.* 23, 2677; Caruthers *et al.,* 1992, *Methods in Enzymology* 211,3-19 (incorporated by reference herein)) have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability, as described above.

In one embodiment, nucleic acid molecules of the invention include one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) G-clamp nucleotides. A G-clamp nucleotide is a modified cytosine analog wherein the modifications confer the ability to hydrogen bond both Watson-Crick and Hoogsteen faces of a complementary guanine within a duplex, see for example Lin and Matteucci, 1998, *J. Am. Chem. Soc.*, 120, 8531-8532. A single G-clamp analog substitution within an oligonucleotide can result in substantially enhanced helical thermal stability and mismatch discrimination when hybridized to complementary oligonucleotides. The inclusion of such nucleotides in nucleic acid molecules of the invention results in both enhanced affinity and specificity to nucleic acid targets, complementary sequences, or template strands. In another embodiment, nucleic acid molecules of the invention include one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) LNA "locked nucleic acid" nucleotides such as a 2', 4'-C methylene bicyclo

nucleotide (see for example Wengel *et al.,* International PCT Publication No. WO 00/66604 and WO 99/14226).

In another embodiment, the invention features conjugates and/or complexes of siNA molecules of the invention. Such conjugates and/or complexes can be used to facilitate delivery of siNA molecules into a biological system, such as a cell. The conjugates and complexes provided by the instant invention can impart therapeutic activity by transferring therapeutic compounds across cellular membranes, altering the pharmacokinetics, and/or modulating the localization of nucleic acid molecules of the invention. The present invention encompasses the design and synthesis of novel conjugates and complexes for the delivery of molecules, including, but not limited to, small molecules, lipids, phospholipids, nucleosides, nucleotides, nucleic acids, antibodies, toxins, negatively charged polymers and other polymers, for example proteins, peptides, hormones, carbohydrates, polyethylene glycols, or polyamines, across cellular membranes. In general, the transporters described are designed to be used either individually or as part of a multi-component system, with or without degradable linkers. These compounds are expected to improve delivery and/or localization of nucleic acid molecules of the invention into a number of cell types originating from different tissues, in the presence or absence of serum (see Sullenger and Cech, U.S. Pat. No. 5,854,038). Conjugates of the molecules described herein can be attached to biologically active molecules via linkers that are biodegradable, such as biodegradable nucleic acid linker molecules.

The term "biodegradable linker" as used herein, refers to a nucleic acid or non-nucleic acid linker molecule that is designed as a biodegradable linker to connect one molecule to another molecule, for example, a biologically active molecule to a siNA molecule of the invention or the sense and antisense strands of a siNA molecule of the invention. The biodegradable linker is designed such that its stability can be modulated for a particular purpose, such as delivery to a particular tissue or cell type. The stability of a nucleic acid-based biodegradable linker molecule can be modulated by using various chemistries, for example combinations of ribonucleotides, deoxyribonucleotides, and chemically-modified nucleotides, such as 2'-O-methyl, 2'-fluoro, 2'-amino, 2'-O-amino, 2'-C-allyl, 2'-O-allyl, and other 2'-modified or base modified nucleotides. The biodegradable nucleic acid linker

molecule can be a dimer, trimer, tetramer or longer nucleic acid molecule, for example, an oligonucleotide of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length, or can comprise a single nucleotide with a phosphorus-based linkage, for example, a phosphoramidate or phosphodiester linkage. The biodegradable nucleic acid linker molecule can also comprise nucleic acid backbone, nucleic acid sugar, or nucleic acid base modifications.

The term "biodegradable" as used herein, refers to degradation in a biological system, for example enzymatic degradation or chemical degradation.

The term "biologically active molecule" as used herein, refers to compounds or molecules that are capable of eliciting or modifying a biological response in a system. Non-limiting examples of biologically active siNA molecules either alone or in combination with other molecules contemplated by the instant invention include therapeutically active molecules such as antibodies, hormones, antivirals, peptides, proteins, chemotherapeutics, small molecules, vitamins, co-factors, nucleosides, nucleotides, oligonucleotides, enzymatic nucleic acids, antisense nucleic acids, triplex forming oligonucleotides, 2,5-A chimeras, siNA, dsRNA, allozymes, aptamers, decoys and analogs thereof. Biologically active molecules of the invention also include molecules capable of modulating the pharmacokinetics and/or pharmacodynamics of other biologically active molecules, for example, lipids and polymers such as polyamines, polyamides, polyethylene glycol and other polyethers.

The term "phospholipid" as used herein, refers to a hydrophobic molecule comprising at least one phosphorus group. For example, a phospholipid can comprise a phosphorus-containing group and saturated or unsaturated alkyl group, optionally substituted with OH, COOH, oxo, amine, or substituted or unsubstituted aryl groups.

Therapeutic nucleic acid molecules (*e.g.*, siNA molecules) delivered exogenously optimally are stable within cells until reverse transcription of the RNA has been modulated long enough to reduce the levels of the RNA transcript. The nucleic acid molecules are resistant to nucleases in order to function as effective intracellular therapeutic agents.

Improvements in the chemical synthesis of nucleic acid molecules described in the instant invention and in the art have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

In yet another embodiment, siNA molecules having chemical modifications that maintain or enhance enzymatic activity of proteins involved in RNAi are provided. Such nucleic acids are also generally more resistant to nucleases than unmodified nucleic acids. Thus, *in vitro* and/or *in vivo* the activity should not be significantly lowered.

Use of the nucleic acid-based molecules of the invention will lead to better treatment of the disease progression by affording the possibility of combination therapies (*e.g.*, multiple siNA molecules targeted to different genes; nucleic acid molecules coupled with known small molecule modulators; or intermittent treatment with combinations of molecules, including different motifs and/or other chemical or biological molecules). The treatment of subjects with siNA molecules can also include combinations of different types of nucleic acid molecules, such as enzymatic nucleic acid molecules (ribozymes), allozymes, antisense, 2,5-A oligoadenylate, decoys, and aptamers.

In another aspect a siNA molecule of the invention comprises one or more 5' and/or a 3'- cap structure, for example on only the sense siNA strand, the antisense siNA strand, or both siNA strands.

By "cap structure" is meant chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see, for example, Adamic *et al.*, U.S. Pat. No. 5,998,203, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and may help in delivery and/or localization within a cell. The cap may be present at the 5'-terminus (5'-cap) or at the 3'-terminal (3'-cap) or may be present on both termini. In non-limiting examples, the 5'-cap is selected from the group consisting of glyceryl, inverted deoxy abasic residue (moiety); 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide; carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; *threo*-pentofuranosyl nucleotide;

acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety.

In non-limiting examples, the 3'-cap is selected from the group consisting of glyceryl, inverted deoxy abasic residue (moiety), 4', 5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate; 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein).

By the term "non-nucleotide" is meant any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine and therefore lacks a base at the 1'-position.

An "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted the substituted

group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino, or SH. The term also includes alkenyl groups that are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably, it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2, halogen, $N(CH_3)_2$, amino, or SH. The term "alkyl" also includes alkynyl groups that have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably, it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino or SH.

Such alkyl groups can also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group that has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

By "nucleotide" as used herein is as recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see, for example, Usman and McSwiggen, *supra*; Eckstein *et al.*, International PCT Publication No. WO 92/07065; Usman *et al.*, International PCT Publication No. WO 93/15187; Uhlman & Peyman, *supra*, all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach *et al.*, 1994, *Nucleic Acids Res.* 22, 2183. Some of the non-limiting examples of base modifications that can be introduced into nucleic acid molecules include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (*e.g.*, 5-methylcytidine), 5-alkyluridines (*e.g.*, ribothymidine), 5-halouridine (*e.g.*, 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (*e.g.* 6-methyluridine), propyne, and others (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090; Uhlman & Peyman, *supra*). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents.

In one embodiment, the invention features modified siNA molecules, with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, phosphotriester, morpholino, amidate carbamate, carboxymethyl, acetamidate, polyamide, sulfonate, sulfonamide, sulfamate, formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications, see Hunziker and Leumann, 1995, *Nucleic Acid Analogues: Synthesis and Properties,* in *Modern Synthetic Methods,* VCH, 331-417, and Mesmaeker *et al.*, 1994, *Novel Backbone Replacements for Oligonucleotides,* in *Carbohydrate Modifications in Antisense Research,* ACS, 24-39.

By "abasic" is meant sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, see for example Adamic *et al.*, U.S. Pat. No. 5,998,203.

By "unmodified nucleoside" is meant one of the bases adenine, cytosine, guanine, thymine, or uracil joined to the 1' carbon of β-D-ribo-furanose.

By "modified nucleoside" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate. Non-limiting examples of modified nucleotides are shown by Formulae I-VII and/or other modifications described herein.

In connection with 2'-modified nucleotides as described for the present invention, by "amino" is meant 2'-NH_2 or 2'-*O*- NH_2, which can be modified or unmodified. Such modified groups are described, for example, in Eckstein *et al.,* U.S. Pat. No. 5,672,695 and Matulic-Adamic *et al.,* U.S. Pat. No. 6,248,878, which are both incorporated by reference in their entireties.

Various modifications to nucleic acid siNA structure can be made to enhance the utility of these molecules. Such modifications will enhance shelf-life, half-life *in vitro,* stability, and ease of introduction of such oligonucleotides to the target site, *e.g.,* to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

Administration of Nucleic Acid Molecules

A siNA molecule of the invention can be adapted for use to treat, for example, tumor angiogenesis and cancer, including but not limited to breast cancer, lung cancer (including non-small cell lung carcinoma), prostate cancer, colorectal cancer, brain cancer, esophageal cancer, bladder cancer, pancreatic cancer, cervical cancer, head and neck cancer, skin cancers, nasopharyngeal carcinoma, liposarcoma, epithelial carcinoma, renal cell carcinoma, gallbladder adeno carcinoma, parotid adenocarcinoma, ovarian cancer, melanoma, lymphoma, glioma, endometrial sarcoma, multidrug resistant cancers, diabetic retinopathy, macular degeneration, neovascular glaucoma, myopic degeneration, arthritis, psoriasis, endometriosis, female reproduction, verruca vulgaris, angiofibroma of tuberous sclerosis, pot-wine stains, Sturge Weber syndrome, Kippel-Trenaunay-Weber syndrome, Osler-Weber-Rendu syndrome, renal disease such as Autosomal dominant polycystic kidney

disease (ADPKD), and any other diseases or conditions that are related to or will respond to the levels of VEGF, VEGFr1, VEGFr2 and/or VEGFr3 in a cell or tissue, alone or in combination with other therapies For example, a siNA molecule can comprise a delivery vehicle, including liposomes, for administration to a subject, carriers and diluents and their salts, and/or can be present in pharmaceutically acceptable formulations. Methods for the delivery of nucleic acid molecules are described in Akhtar *et al.*, 1992, *Trends Cell Bio.*, 2, 139; *Delivery Strategies for Antisense Oligonucleotide Therapeutics*, ed. Akhtar, 1995, Maurer *et al.*, 1999, *Mol. Membr. Biol.*, 16, 129-140; Hofland and Huang, 1999, *Handb. Exp. Pharmacol.*, 137, 165-192; and Lee *et al.*, 2000, *ACS Symp. Ser.*, 752, 184-192, all of which are incorporated herein by reference. Beigelman *et al.*, U.S. Pat. No. 6,395,713 and Sullivan *et al.*, PCT WO 94/02595 further describe the general methods for delivery of nucleic acid molecules. These protocols can be utilized for the delivery of virtually any nucleic acid molecule. Nucleic acid molecules can be administered to cells by a variety of methods known to those of skill in the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins (see for example Gonzalez *et al.*, 1999, *Bioconjugate Chem.*, 10, 1068-1074), biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors (O'Hare and Normand, International PCT Publication No. WO 00/53722). Alternatively, the nucleic acid/vehicle combination is locally delivered by direct injection or by use of an infusion pump. Direct injection of the nucleic acid molecules of the invention, whether subcutaneous, intramuscular, or intradermal, can take place using standard needle and syringe methodologies, or by needle-free technologies such as those described in Conry *et al.*, 1999, *Clin. Cancer Res.*, 5, 2330-2337 and Barry *et al.*, International PCT Publication No. WO 99/31262. The molecules of the instant invention can be used as pharmaceutical agents. Pharmaceutical agents prevent, modulate the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state in a subject.

Thus, the invention features a pharmaceutical composition comprising one or more nucleic acid(s) of the invention in an acceptable carrier, such as a stabilizer, buffer, and the like. The polynucleotides of the invention can be administered (*e.g.*, RNA, DNA or protein) and introduced into a subject by any standard means, with or without stabilizers, buffers, and

the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be followed. The compositions of the present invention can also be formulated and used as tablets, capsules or elixirs for oral administration, suppositories for rectal administration, sterile solutions, suspensions for injectable administration, and the other compositions known in the art.

The present invention also includes pharmaceutically acceptable formulations of the compounds described. These formulations include salts of the above compounds, *e.g.*, acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, *e.g.*, systemic administration, into a cell or subject, including for example a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation from reaching a target cell (*i.e.*, a cell to which the negatively charged nucleic acid is desirable for delivery). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms that prevent the composition or formulation from exerting its effect.

By "systemic administration" is meant *in vivo* systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes that lead to systemic absorption include, without limitation: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes exposes the siNA molecules of the invention to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation that can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach can

provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells, such as cells producing excess VEGF and/or VEGFr.

By "pharmaceutically acceptable formulation" is meant, a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the instant invention in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the instant invention include: P-glycoprotein inhibitors (such as Pluronic P85), which can enhance entry of drugs into the CNS (Jolliet-Riant and Tillement, 1999, *Fundam. Clin. Pharmacol.*, 13, 16-26); biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery after intracerebral implantation (Emerich, DF *et al*, 1999, *Cell Transplant*, 8, 47-58) (Alkermes, Inc. Cambridge, MA); and loaded nanoparticles, such as those made of polybutylcyanoacrylate, which can deliver drugs across the blood brain barrier and can alter neuronal uptake mechanisms (*Prog Neuropsychopharmacol Biol Psychiatry*, 23, 941-949, 1999). Other non-limiting examples of delivery strategies for the nucleic acid molecules of the instant invention include material described in Boado *et al.*, 1998, *J. Pharm. Sci.*, 87, 1308-1315; Tyler *et al.*, 1999, *FEBS Lett.*, 421, 280-284; Pardridge *et al.*, 1995, *PNAS USA.*, 92, 5592-5596; Boado, 1995, *Adv. Drug Delivery Rev.*, 15, 73-107; Aldrian-Herrada *et al.*, 1998, *Nucleic Acids Res.*, 26, 4910-4916; and Tyler *et al.*, 1999, *PNAS USA.*, 96, 7053-7058.

The invention also features the use of the composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic *et al. Chem. Rev.* 1995, 95, 2601-2627; Ishiwata *et al., Chem. Pharm. Bull.* 1995, 43, 1005-1011). Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic *et al., Science* 1995, 267, 1275-1276; Oku *et al.,*1995, *Biochim. Biophys. Acta*, 1238, 86-90). The long-

circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu *et al., J. Biol. Chem.* 1995, 42, 24864-24870; Choi *et al.,* International PCT Publication No. WO 96/10391; Ansell *et al.,* International PCT Publication No. WO 96/10390; Holland *et al.,* International PCT Publication No. WO 96/10392). Long-circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

The present invention also includes compositions prepared for storage or administration that include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985), hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors that those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

The nucleic acid molecules of the invention and formulations thereof can be administered orally, topically, parenterally, by inhalation or spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and/or vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (*e.g.*, intravenous), intramuscular, or intrathecal injection or

infusion techniques and the like. In addition, there is provided a pharmaceutical formulation comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active ingredients. The pharmaceutical compositions containing nucleic acid molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients can be, for example, inert diluents; such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate can be employed.

Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in a mixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-methylcellulose,

sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present.

Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for

example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The nucleic acid molecules of the invention can also be administered in the form of suppositories, *e.g.*, for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

Nucleic acid molecules of the invention can be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per subject per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the host treated

and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

It is understood that the specific dose level for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

The nucleic acid molecules of the present invention can also be administered to a subject in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

In one embodiment, the invention comprises compositions suitable for administering nucleic acid molecules of the invention to specific cell types. For example, the asialoglycoprotein receptor (ASGPr) (Wu and Wu, 1987, *J. Biol. Chem.* 262, 4429-4432) is unique to hepatocytes and binds branched galactose-terminal glycoproteins, such as asialoorosomucoid (ASOR). In another example, the folate receptor is overexpressed in many cancer cells. Binding of such glycoproteins, synthetic glycoconjugates, or folates to the receptor takes place with an affinity that strongly depends on the degree of branching of the oligosaccharide chain, for example, triatennary structures are bound with greater affinity than biatenarry or monoatennary chains (Baenziger and Fiete, 1980, *Cell,* 22, 611-620; Connolly *et al.,* 1982, *J. Biol. Chem.,* 257, 939-945). Lee and Lee, 1987, *Glycoconjugate J.,* 4, 317-328, obtained this high specificity through the use of N-acetyl-D-galactosamine as the carbohydrate moiety, which has higher affinity for the receptor, compared to galactose. This "clustering effect" has also been described for the binding and uptake of mannosyl-

terminating glycoproteins or glycoconjugates (Ponpipom *et al.*, 1981, *J. Med. Chem.*, 24, 1388-1395). The use of galactose, galactosamine, or folate based conjugates to transport exogenous compounds across cell membranes can provide a targeted delivery approach to, for example, the treatment of liver disease, cancers of the liver, or other cancers. The use of bioconjugates can also provide a reduction in the required dose of therapeutic compounds required for treatment. Furthermore, therapeutic bioavialability, pharmacodynamics, and pharmacokinetic parameters can be modulated through the use of nucleic acid bioconjugates of the invention. Non-limiting examples of such bioconjugates are described in Vargeese *et al.*, USSN 10/201,394, filed August 13, 2001; and Matulic-Adamic *et al.*, USSN 60/362,016, filed March 6, 2002.

Alternatively, certain siNA molecules of the instant invention can be expressed within cells from eukaryotic promoters (*e.g.*, Izant and Weintraub, 1985, *Science*, 229, 345; McGarry and Lindquist, 1986, *Proc. Natl. Acad. Sci.*, USA 83, 399; Scanlon *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, 88, 10591-5; Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Dropulic *et al.*, 1992, *J. Virol.*, 66, 1432-41; Weerasinghe *et al.*, 1991, *J. Virol.*, 65, 5531-4; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Sarver *et al.*, 1990 *Science*, 247, 1222-1225; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Good *et al.*, 1997, *Gene Therapy*, 4, 45. Those skilled in the art realize that any nucleic acid can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by a enzymatic nucleic acid (Draper *et al.*, PCT WO 93/23569, and Sullivan *et al.*, PCT WO 94/02595; Ohkawa *et al.*, 1992, *Nucleic Acids Symp. Ser.*, 27, 15-6; Taira *et al.*, 1991, *Nucleic Acids Res.*, 19, 5125-30; Ventura *et al.*, 1993, *Nucleic Acids Res.*, 21, 3249-55; Chowrira *et al.*, 1994, *J. Biol. Chem.*, 269, 25856.

In another aspect of the invention, RNA molecules of the present invention can be expressed from transcription units (see for example Couture *et al.*, 1996, *TIG.*, 12, 510) inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. In another embodiment, pol III based constructs are used to express nucleic acid molecules of the invention (see for

example Thompson, U.S. Pats. Nos. 5,902,880 and 6,146,886). The recombinant vectors capable of expressing the siNA molecules can be delivered as described above, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of nucleic acid molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecule interacts with the target mRNA and generates an RNAi response. Delivery of siNA molecule expressing vectors can be systemic, such as by intravenous or intra-muscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell (for a review see Couture *et al.,* 1996, *TIG.,* 12, 510).

In one aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the instant invention. The expression vector can encode one or both strands of a siNA duplex, or a single self-complementary strand that self hybridizes into a siNA duplex. The nucleic acid sequences encoding the siNA molecules of the instant invention can be operably linked in a manner that allows expression of the siNA molecule (see for example Paul *et al.,* 2002, *Nature Biotechnology,* 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology,* 19, 497; Lee *et al.,* 2002, *Nature Biotechnology,* 19, 500; and Novina *et al.,* 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725).

In another aspect, the invention features an expression vector comprising: a) a transcription initiation region (*e.g.,* eukaryotic pol I, II or III initiation region); b) a transcription termination region (*e.g.,* eukaryotic pol I, II or III termination region); and c) a nucleic acid sequence encoding at least one of the siNA molecules of the instant invention,wherein said sequence is operably linked to said initiation region and said termination region in a manner that allows expression and/or delivery of the siNA molecule. The vector can optionally include an open reading frame (ORF) for a protein operably linked on the 5' side or the 3'-side of the sequence encoding the siNA of the invention; and/or an intron (intervening sequences).

Transcription of the siNA molecule sequences can be driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters are expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type depends on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990, *Proc. Natl. Acad. Sci. U S A*, 87, 6743-7; Gao and Huang 1993, *Nucleic Acids Res*., 21, 2867-72; Lieber *et al.*, 1993, *Methods Enzymol.*, 217, 47-66; Zhou *et al.*, 1990, *Mol. Cell. Biol*., 10, 4529-37). Several investigators have demonstrated that nucleic acid molecules expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. U S A*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res*., 20, 4581-9; Yu *et al.*, 1993, *Proc. Natl. Acad. Sci. U S A*, 90, 6340-4; L'Huillier *et al.*, 1992, *EMBO J.*, 11, 4411-8; Lisziewicz *et al.*, 1993, *Proc. Natl. Acad. Sci. U. S. A*, 90, 8000-4; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Sullenger & Cech, 1993, *Science*, 262, 1566). More specifically, transcription units such as the ones derived from genes encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as siNA in cells (Thompson *et al., supra*; Couture and Stinchcomb, *1996, supra*; Noonberg *et al.*, 1994, *Nucleic Acid Res.*, 22, 2830; Noonberg *et al.*, U.S. Pat. No. 5,624,803; Good *et al.*, 1997, *Gene Ther.*, 4, 45; Beigelman *et al.*, International PCT Publication No. WO 96/18736. The above siNA transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, *1996, supra)*.

In another aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the siNA molecules of the invention in a manner that allows expression of that siNA molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; and c)

a nucleic acid sequence encoding at least one strand of the siNA molecule, wherein the sequence is operably linked to the initiation region and the termination region in a manner that allows expression and/or delivery of the siNA molecule.

In another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; and d) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the open reading frame and the termination region in a manner that allows expression and/or delivery of the siNA molecule. In yet another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; and d) a nucleic acid sequence encoding at least one siNA molecule, wherein the sequence is operably linked to the initiation region, the intron and the termination region in a manner which allows expression and/or delivery of the nucleic acid molecule.

In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; and e) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the intron, the open reading frame and the termination region in a manner which allows expression and/or delivery of the siNA molecule.

<u>VEGF/VEGFr biology and biochemistry</u>

The following discussion is adapted from R&D Systems, Cytokine Mini Reviews, Vascular Endothelial Growth Factor (VEGF), Copyright ©2002 R&D Systems. Angiogenesis is a process of new blood vessel development from pre-existing vasculature. It plays an essential role in embryonic development, normal growth of tissues, wound healing, the female reproductive cycle (i.e., ovulation, menstruation and placental development), as well as a major role in many diseases. Particular interest has focused on cancer, since

tumors cannot grow beyond a few millimeters in size without developing a new blood supply. Angiogenesis is also necessary for the spread and growth of tumor cell metastases.

One of the most important growth and survival factors for endothelium is vascular endothelial growth factor (VEGF). VEGF induces angiogenesis and endothelial cell proliferation and plays an important role in regulating vasculogenesis. VEGF is a heparin-binding glycoprotein that is secreted as a homodimer of 45 kDa. Most types of cells, but usually not endothelial cells themselves, secrete VEGF. Since the initially discovered VEGF, VEGF-A, increases vascular permeability, it was known as vascular permeability factor. In addition, VEGF causes vasodilatation, partly through stimulation of nitric oxide synthase in endothelial cells. VEGF can also stimulate cell migration and inhibit apoptosis.

There are several splice variants of VEGF-A. The major ones include: 121, 165, 189 and 206 amino acids (aa), each one comprising a specific exon addition. VEGF165 is the most predominant protein, but transcripts of VEGF 121 may be more abundant. VEGF206 is rarely expressed and has been detected only in fetal liver. Recently, other splice variants of 145 and 183 aa have also been described. The 165, 189 and 206 aa splice variants have heparin-binding domains, which help anchor them in extracellular matrix and are involved in binding to heparin sulfate and presentation to VEGF receptors. Such presentation is a key factor for VEGF potency (i.e., the heparin-binding forms are more active). Several other members of the VEGF family have been cloned including VEGF-B, -C, and -D. Placenta growth factor (PlGF) is also closely related to VEGF-A. VEGF-A, -B, -C, -D, and PlGF are all distantly related to platelet-derived growth factors-A and -B. Less is known about the function and regulation of VEGF-B, -C, and -D, but they do not seem to be regulated by the major pathways that regulate VEGF-A.

VEGF-A transcription is potentiated in response to hypoxia and by activated oncogenes. The transcription factors, hypoxia inducible factor-1a (hif-1a) and -2a, are degraded by proteosomes in normoxia and stabilized in hypoxia. This pathway is dependent on the Von Hippel-Lindau gene product. Hif-1a and hif-2 a heterodimerize with the aryl hydrocarbon nuclear translocator in the nucleus and bind the VEGF promoter/enhancer. This is a key pathway expressed in most types of cells. Hypoxia inducibility, in particular,

characterizes VEGF-A versus other members of the VEGF family and other angiogenic factors. VEGF transcription in normoxia is activated by many oncogenes, including H-ras and several transmembrane tyrosine kinases, such as the epidermal growth factor receptor and erbB2. These pathways together account for a marked upregulation of VEGF-A in tumors compared to normal tissues and are often of prognostic importance.

There are three receptors in the VEGF receptor family. They have the common properties of multiple IgG-like extracellular domains and tyrosine kinase activity. The enzyme domains of VEGF receptor 1 (VEGFr1, also known as Flt-1), VEGFr2 (also known as KDR or Flk-1), and VEGFr3 (also known as Flt-4) are divided by an inserted sequence. Endothelial cells also express additional VEGF receptors, Neuropilin-1 and Neuropilin-2. VEGF-A binds to VEGFr1 and VEGFr2 and to Neuropilin-1 and Neuropilin-2. PlGF and VEGF-B bind VEGFr1 and Neuropilin-1. VEGF-C and -D bind VEGFr3 and VEGFr2.

The VEGF-C/VEGFr3 pathway is important for lymphatic proliferation. VEGFr3 is specifically expressed on lymphatic endothelium. A soluble form of Flt-1 can be detected in peripheral blood and is a high affinity ligand for VEGF. Soluble Flt-1 can be used to antagonize VEGF function. VEGFr1 and VEGFr2 are upregulated in tumor and proliferating endothelium, partly by hypoxia and also in response to VEGF-A itself. VEGFr1 and VEGFr2 can interact with multiple downstream signaling pathways via proteins such as PLC-g, Ras, Shc, Nck, PKC and PI3-kinase. VEGFr1 is of higher affinity than VEGFr2 and mediates motility and vascular permeability. VEGFr2 is necessary for proliferation.

VEGF can be detected in both plasma and serum samples of patients, with much higher levels in serum. Platelets release VEGF upon aggregation and may be a major source of VEGF delivery to tumors. Several studies have shown that association of high serum levels of VEGF with poor prognosis in cancer patients may be correlated with an elevated platelet count. Many tumors release cytokines that can stimulate the production of megakaryocytes in the marrow and elevate the platelet count. This can result in an indirect increase of VEGF delivery to tumors.

VEGF is implicated in several other pathological conditions associated with enhanced angiogenesis. For example, VEGF plays a role in both psoriasis and rheumatoid arthritis. Diabetic retinopathy is associated with high intraocular levels of VEGF. Inhibition of VEGF function may result in infertility by blockade of corpus luteum function. Direct demonstration of the importance of VEGF in tumor growth has been achieved using dominant negative VEGF receptors to block in vivo proliferation, as well as blocking antibodies to VEGF39 or to VEGFr2.

The use of small interfering nucleic acid molecules targeting VEGF and corresponding receptors and ligands therefore provides a class of novel therapeutic agents that can be used in the diagnosis of and the treatment of cancer, proliferative diseases, or any other disease or condition that responds to modulation of VEGF and/or VEGFr genes.

Examples:

The following are non-limiting examples showing the selection, isolation, synthesis and activity of nucleic acids of the instant invention.

Example 1: Tandem synthesis of siNA constructs

Exemplary siNA molecules of the invention are synthesized in tandem using a cleavable linker, for example, a succinyl-based linker. Tandem synthesis as described herein is followed by a one-step purification process that provides RNAi molecules in high yield. This approach is highly amenable to siNA synthesis in support of high throughput RNAi screening, and can be readily adapted to multi-column or multi-well synthesis platforms.

After completing a tandem synthesis of a siNA oligo and its complement in which the 5'-terminal dimethoxytrityl (5'-O-DMT) group remains intact (trityl on synthesis), the oligonucleotides are deprotected as described above. Following deprotection, the siNA sequence strands are allowed to spontaneously hybridize. This hybridization yields a duplex in which one strand has retained the 5'-O-DMT group while the complementary strand comprises a terminal 5'-hydroxyl. The newly formed duplex behaves as a single molecule

during routine solid-phase extraction purification (Trityl-On purification) even though only one molecule has a dimethoxytrityl group. Because the strands form a stable duplex, this dimethoxytrityl group (or an equivalent group, such as other trityl groups or other hydrophobic moieties) is all that is required to purify the pair of oligos, for example, by using a C18 cartridge.

Standard phosphoramidite synthesis chemistry is used up to the point of introducing a tandem linker, such as an inverted deoxy abasic succinate or glyceryl succinate linker (see **Figure 1**) or an equivalent cleavable linker. A non-limiting example of linker coupling conditions that can be used includes a hindered base such as diisopropylethylamine (DIPA) and/or DMAP in the presence of an activator reagent such as Bromotripyrrolidinophosphoniumhexaflurorophosphate (PyBrOP). After the linker is coupled, standard synthesis chemistry is utilized to complete synthesis of the second sequence leaving the terminal the 5'-O-DMT intact. Following synthesis, the resulting oligonucleotide is deprotected according to the procedures described herein and quenched with a suitable buffer, for example with 50mM NaOAc or 1.5M $NH_4H_2CO_3$.

Purification of the siNA duplex can be readily accomplished using solid phase extraction, for example using a Waters C18 SepPak 1g cartridge conditioned with 1 column volume (CV) of acetonitrile, 2 CV H2O, and 2 CV 50mM NaOAc. The sample is loaded and then washed with 1 CV H2O or 50mM NaOAc. Failure sequences are eluted with 1 CV 14% ACN (Aqueous with 50mM NaOAc and 50mM NaCl). The column is then washed, for example with 1 CV H2O followed by on-column detritylation, for example by passing 1 CV of 1% aqueous trifluoroacetic acid (TFA) over the column, then adding a second CV of 1% aqueous TFA to the column and allowing to stand for approximately 10 minutes. The remaining TFA solution is removed and the column washed with H20 followed by 1 CV 1M NaCl and additional H2O. The siNA duplex product is then eluted, for example, using 1 CV 20% aqueous CAN.

Figure 2 provides an example of MALDI-TOV mass spectrometry analysis of a purified siNA construct in which each peak corresponds to the calculated mass of an individual siNA strand of the siNA duplex. The same purified siNA provides three peaks

when analyzed by capillary gel electrophoresis (CGE), one peak presumably corresponding to the duplex siNA, and two peaks presumably corresponding to the separate siNA sequence strands. Ion exchange HPLC analysis of the same siNA contract only shows a single peak. Testing of the purified siNA construct using a luciferase reporter assay described below demonstrated the same RNAi activity compared to siNA constructs generated from separately synthesized oligonucleotide sequence strands.

Example 2: Identification of potential siNA target sites in any RNA sequence

The sequence of an RNA target of interest, such as a viral or human mRNA transcript, is screened for target sites, for example by using a computer folding algorithm. In a non-limiting example, the sequence of a gene or RNA gene transcript derived from a database, such as Genbank, is used to generate siNA targets having complementarity to the target. Such sequences can be obtained from a database, or can be determined experimentally as known in the art. Target sites that are known, for example, those target sites determined to be effective target sites based on studies with other nucleic acid molecules, for example ribozymes or antisense, or those targets known to be associated with a disease or condition such as those sites containing mutations or deletions, can be used to design siNA molecules targeting those sites. Various parameters can be used to determine which sites are the most suitable target sites within the target RNA sequence. These parameters include but are not limited to secondary or tertiary RNA structure, the nucleotide base composition of the target sequence, the degree of homology between various regions of the target sequence, or the relative position of the target sequence within the RNA transcript. Based on these determinations, any number of target sites within the RNA transcript can be chosen to screen siNA molecules for efficacy, for example by using *in vitro* RNA cleavage assays, cell culture, or animal models. In a non-limiting example, anywhere from 1 to 1000 target sites are chosen within the transcript based on the size of the siNA construct to be used. High throughput screening assays can be developed for screening siNA molecules using methods known in the art, such as with multi-well or multi-plate assays to determine efficient reduction in target gene expression.

Example 3: Selection of siNA molecule target sites in a RNA

The following non-limiting steps can be used to carry out the selection of siNAs targeting a given gene sequence or transcript.

1. The target sequence is parsed *in silico* into a list of all fragments or subsequences of a particular length, for example 23 nucleotide fragments, contained within the target sequence. This step is typically carried out using a custom Perl script, but commercial sequence analysis programs such as Oligo, MacVector, or the GCG Wisconsin Package can be employed as well.

2. In some instances the siNAs correspond to more than one target sequence; such would be the case for example in targeting different transcripts of the same gene, targeting different transcripts of more than one gene, or for targeting both the human gene and an animal homolog. In this case, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find matching sequences in each list. The subsequences are then ranked according to the number of target sequences that contain the given subsequence; the goal is to find subsequences that are present in most or all of the target sequences. Alternately, the ranking can identify subsequences that are unique to a target sequence, such as a mutant target sequence. Such an approach would enable the use of siNA to target specifically the mutant sequence and not effect the expression of the normal sequence.

3. In some instances the siNA subsequences are absent in one or more sequences while present in the desired target sequence; such would be the case if the siNA targets a gene with a paralogous family member that is to remain untargeted. As in case 2 above, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find sequences that are present in the target gene but are absent in the untargeted paralog.

4. The ranked siNA subsequences can be further analyzed and ranked according to GC content. A preference can be given to sites containing 30-70% GC, with a further preference to sites containing 40-60% GC.

5. The ranked siNA subsequences can be further analyzed and ranked according to self-folding and internal hairpins. Weaker internal folds are preferred; strong hairpin structures are to be avoided.

6. The ranked siNA subsequences can be further analyzed and ranked according to whether they have runs of GGG or CCC in the sequence. GGG (or even more Gs) in either strand can make oligonucleotide synthesis problematic and can potentially interfere with RNAi activity, so it is avoided whenever better sequences are available. CCC is searched in the target strand because that will place GGG in the antisense strand.

7. The ranked siNA subsequences can be further analyzed and ranked according to whether they have the dinucleotide UU (uridine dinucleotide) on the 3'-end of the sequence, and/or AA on the 5'-end of the sequence (to yield 3' UU on the antisense sequence). These sequences allow one to design siNA molecules with terminal TT thymidine dinucleotides.

8. Four or five target sites are chosen from the ranked list of subsequences as described above. For example, in subsequences having 23 nucleotides, the right 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the upper (sense) strand of the siNA duplex, while the reverse complement of the left 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the lower (antisense) strand of the siNA duplex (see **Tables II and III**). If terminal TT residues are desired for the sequence (as described in paragraph 7), then the two 3' terminal nucleotides of both the sense and antisense strands are replaced by TT prior to synthesizing the oligos.

9. The siNA molecules are screened in an *in vitro*, cell culture or animal model system to identify the most active siNA molecule or the most preferred target site within the target RNA sequence.

In an alternate approach, a pool of siNA constructs specific to a VEGF and/or VEGFr target sequence is used to screen for target sites in cells expressing VEGF and/or VEGFr RNA, such as HUVEC, HMVEC, or A375 cells. The general strategy used in this approach

is shown in **Figure 9.** A non-limiting example of such is a pool comprising sequences having any of SEQ ID NOS 1-2238. Cells expressing VEGF and/or VEGFr (e.g., HUVEC, HMVEC, or A375 cells) are transfected with the pool of siNA constructs and cells that demonstrate a phenotype associated with VEGF and/or VEGFr inhibition are sorted. The pool of siNA constructs can be expressed from transcription cassettes inserted into appropriate vectors (see for example **Figure 7** and **Figure 8**). The siNA from cells demonstrating a positive phenotypic change (e.g., decreased proliferation, decreased VEGF and/or VEGFr mRNA levels or decreased VEGF and/or VEGFr protein expression), are sequenced to determine the most suitable target site(s) within the target VEGF and/or VEGFr RNA sequence.

Example 4: VEGF and/or VEGFr targeted siNA design

siNA target sites were chosen by analyzing sequences of the VEGF and/or VEGFr RNA target and optionally prioritizing the target sites on the basis of folding (structure of any given sequence analyzed to determine siNA accessibility to the target), by using a library of siNA molecules as described in Example 3, or alternately by using an *in vitro* siNA system as described in Example 6 herein. siNA molecules were designed that could bind each target and are optionally individually analyzed by computer folding to assess whether the siNA molecule can interact with the target sequence. Varying the length of the siNA molecules can be chosen to optimize activity. Generally, a sufficient number of complementary nucleotide bases are chosen to bind to, or otherwise interact with, the target RNA, but the degree of complementarity can be modulated to accommodate siNA duplexes or varying length or base composition. By using such methodologies, siNA molecules can be designed to target sites within any known RNA sequence, for example those RNA sequences corresponding to the any gene transcript.

Chemically modified siNA constructs are designed to provide nuclease stability for systemic administration in vivo and/or improved pharmacokinetic, localization, and delivery properties while preserving the ability to mediate RNAi activity. Chemical modifications as described herein are introduced synthetically using synthetic methods described herein and those generally known in the art. The synthetic siNA constructs are then assayed for

nuclease stability in serum and/or cellular/tissue extracts (e.g. liver extracts). The synthetic siNA constructs are also tested in parallel for RNAi activity using an appropriate assay, such as a luciferase reporter assay as described herein or another suitable assay that can quantity RNAi activity. Synthetic siNA constructs that possess both nuclease stability and RNAi activity can be further modified and re-evaluated in stability and activity assays. The chemical modifications of the stabilized active siNA constructs can then be applied to any siNA sequence targeting any chosen RNA and used, for example, in target screening assays to pick lead siNA compounds for therapeutic development (see for example **Figure 11**).

Example 5: Chemical Synthesis and Purification of siNA

siNA molecules can be designed to interact with various sites in the RNA message, for example, target sequences within the RNA sequences described herein. The sequence of one strand of the siNA molecule(s) is complementary to the target site sequences described above. The siNA molecules can be chemically synthesized using methods described herein. Inactive siNA molecules that are used as control sequences can be synthesized by scrambling the sequence of the siNA molecules such that it is not complementary to the target sequence. Generally, siNA constructs can by synthesized using solid phase oligonucleotide synthesis methods as described herein (see for example Usman *et al.,* US Patent Nos. 5,804,683; 5,831,071; 5,998,203; 6,117,657; 6,353,098; 6,362,323; 6,437,117; 6,469,158; Scaringe *et al.,* US Patent Nos. 6,111,086; 6,008,400; 6,111,086 all incorporated by reference herein in their entirety).

In a non-limiting example, RNA oligonucleotides are synthesized in a stepwise fashion using the phosphoramidite chemistry as is known in the art. Standard phosphoramidite chemistry involves the use of nucleosides comprising any of 5'-O-dimethoxytrityl, 2'-O-tert-butyldimethylsilyl, 3'-O-2-Cyanoethyl N,N-diisopropylphos-phoroamidite groups, and exocyclic amine protecting groups (e.g. N6-benzoyl adenosine, N4 acetyl cytidine, and N2-isobutyryl guanosine). Alternately, 2'-O-Silyl Ethers can be used in conjunction with acid-labile 2'-O-orthoester protecting groups in the synthesis of RNA as described by Scaringe *supra*. Differing 2' chemistries can require different protecting groups, for example 2'-deoxy-2'-amino nucleosides can utilize N-phthaloyl

protection as described by Usman *et al.,* US Patent 5,631,360, incorporated by reference herein in its entirety).

During solid phase synthesis, each nucleotide is added sequentially (3′- to 5′-direction) to the solid support-bound oligonucleotide. The first nucleoside at the 3′-end of the chain is covalently attached to a solid support (e.g., controlled pore glass or polystyrene) using various linkers. The nucleotide precursor, a ribonucleoside phosphoramidite, and activator are combined resulting in the coupling of the second nucleoside phosphoramidite onto the 5′-end of the first nucleoside. The support is then washed and any unreacted 5′-hydroxyl groups are capped with a capping reagent such as acetic anhydride to yield inactive 5′-acetyl moieties. The trivalent phosphorus linkage is then oxidized to a more stable phosphate linkage. At the end of the nucleotide addition cycle, the 5′-O-protecting group is cleaved under suitable conditions (e.g., acidic conditions for trityl-based groups and Fluoride for silyl-based groups). The cycle is repeated for each subsequent nucleotide.

Modification of synthesis conditions can be used to optimize coupling efficiency, for example by using differing coupling times, differing reagent/phosphoramidite concentrations, differing contact times, differing solid supports and solid support linker chemistries depending on the particular chemical composition of the siNA to be synthesized. Deprotection and purification of the siNA can be performed as is generally described in Deprotection and purification of the siNA can be performed as is generally described in Usman *et al.,* US 5,831,071, US 6,353,098, US 6,437,117, and Bellon *et al.,* US 6,054,576, US 6,162,909, US 6,303,773, or Scaringe *supra,* incorporated by reference herein in their entireties. Additionally, deprotection conditions can be modified to provide the best possible yield and purity of siNA constructs. For example, applicant has observed that oligonucleotides comprising 2'-deoxy-2'-fluoro nucleotides can degrade under inappropriate deprotection conditions. Such oligonucleotides are deprotected using aqueous methylamine at about 35°C for 30 minutes. If the 2'-deoxy-2'-fluoro containing oligonucleotide also comprises ribonucleotides, after deprotection with aqueous methylamine at about 35°C for 30 minutes, TEA-HF is added and the reaction maintained at about 65°C for an additional 15 minutes.

Example 6: RNAi *in vitro* assay to assess siNA activity

An *in vitro* assay that recapitulates RNAi in a cell-free system is used to evaluate siNA constructs targeting VEGF and/or VEGFr RNA targets. The assay comprises the system described by Tuschl *et al.*, 1999, *Genes and Development*, 13, 3191-3197 and Zamore *et al.*, 2000, *Cell*, 101, 25-33 adapted for use with VEGF and/or VEGFr target RNA. A Drosophila extract derived from syncytial blastoderm is used to reconstitute RNAi activity *in vitro*. Target RNA is generated via *in vitro* transcription from an appropriate VEGF and/or VEGFr expressing plasmid using T7 RNA polymerase or via chemical synthesis as described herein. Sense and antisense siNA strands (for example 20 uM each) are annealed by incubation in buffer (such as 100 mM potassium acetate, 30 mM HEPES-KOH, pH 7.4, 2 mM magnesium acetate) for 1 min. at 90°C followed by 1 hour at 37°C , then diluted in lysis buffer (for example 100 mM potassium acetate, 30 mM HEPES-KOH at pH 7.4, 2mM magnesium acetate). Annealing can be monitored by gel electrophoresis on an agarose gel in TBE buffer and stained with ethidium bromide. The Drosophila lysate is prepared using zero to two-hour-old embryos from Oregon R flies collected on yeasted molasses agar that are dechorionated and lysed. The lysate is centrifuged and the supernatant isolated. The assay comprises a reaction mixture containing 50% lysate [vol/vol], RNA (10-50 pM final concentration), and 10% [vol/vol] lysis buffer containing siNA (10 nM final concentration). The reaction mixture also contains 10 mM creatine phosphate, 10 ug.ml creatine phosphokinase, 100 um GTP, 100 uM UTP, 100 uM CTP, 500 uM ATP, 5 mM DTT, 0.1 U/uL RNasin (Promega), and 100 uM of each amino acid. The final concentration of potassium acetate is adjusted to 100 mM. The reactions are pre-assembled on ice and preincubated at 25° C for 10 minutes before adding RNA, then incubated at 25° C for an additional 60 minutes. Reactions are quenched with 4 volumes of 1.25 x Passive Lysis Buffer (Promega). Target RNA cleavage is assayed by RT-PCR analysis or other methods known in the art and are compared to control reactions in which siNA is omitted from the reaction.

Alternately, internally-labeled target RNA for the assay is prepared by *in vitro* transcription in the presence of [alpha-^{32}p] CTP, passed over a G 50 Sephadex column by spin chromatography and used as target RNA without further purification. Optionally,

target RNA is 5'-^{32}P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed as described above and target RNA and the specific RNA cleavage products generated by RNAi are visualized on an autoradiograph of a gel. The percentage of cleavage is determined by Phosphor Imager® quantitation of bands representing intact control RNA or RNA from control reactions without siNA and the cleavage products generated by the assay.

In one embodiment, this assay is used to determine target sites the VEGF and/or VEGFr RNA target for siNA mediated RNAi cleavage, wherein a plurality of siNA constructs are screened for RNAi mediated cleavage of the VEGF and/or VEGFr RNA target, for example, by analyzing the assay reaction by electrophoresis of labeled target RNA, or by northern blotting, as well as by other methodology well known in the art.

Example 7: Nucleic acid inhibition of VEGF and/or VEGFr target RNA *in vivo*

siNA molecules targeted to the huma VEGF and/or VEGFr RNA are designed and synthesized as described above. These nucleic acid molecules can be tested for cleavage activity *in vivo*, for example, using the following procedure. The target sequences and the nucleotide location within the VEGF and/or VEGFr RNA are given in **Table II and III**.

Two formats are used to test the efficacy of siNAs targeting VEGF and/or VEGFr. First, the reagents are tested in cell culture using, for example, HUVEC, HMVEC, or A375 cells to determine the extent of RNA and protein inhibition. siNA reagents (*e.g.*; see **Tables II and III**) are selected against the VEGF and/or VEGFr target as described herein. RNA inhibition is measured after delivery of these reagents by a suitable transfection agent to, for example, HUVEC, HMVEC, or A375 cells. Relative amounts of target RNA are measured versus actin using real-time PCR monitoring of amplification (*eg.*, ABI 7700 Taqman®). A comparison is made to a mixture of oligonucleotide sequences made to unrelated targets or to a randomized siNA control with the same overall length and chemistry, but randomly substituted at each position. Primary and secondary lead reagents are chosen for the target and optimization performed. After an optimal transfection agent concentration is chosen, a

RNA time-course of inhibition is performed with the lead siNA molecule. In addition, a cell-plating format can be used to determine RNA inhibition.

Delivery of siNA to Cells

Cells (e.g., HUVEC, HMVEC, or A375 cells) are seeded, for example, at 1×10^5 cells per well of a six-well dish in EGM-2 (BioWhittaker) the day before transfection. siNA (final concentration, for example 20nM) and cationic lipid (e.g., final concentration 2μg/ml) are complexed in EGM basal media (Biowhittaker) at 37°C for 30 mins in polystyrene tubes. Following vortexing, the complexed siNA is added to each well and incubated for the times indicated. For initial optimization experiments, cells are seeded, for example, at 1×10^3 in 96 well plates and siNA complex added as described. Efficiency of delivery of siNA to cells is determined using a fluorescent siNA complexed with lipid. Cells in 6-well dishes are incubated with siNA for 24 hours, rinsed with PBS and fixed in 2% paraformaldehyde for 15 minutes at room temperature. Uptake of siNA is visualized using a fluorescent microscope.

Taqman and Lightcycler quantification of mRNA

Total RNA is prepared from cells following siNA delivery, for example, using Qiagen RNA purification kits for 6-well or Rneasy extraction kits for 96-well assays. For Taqman analysis, dual-labeled probes are synthesized with the reporter dye, FAM or JOE, covalently linked at the 5'-end and the quencher dye TAMRA conjugated to the 3'-end. One-step RT-PCR amplifications are performed on, for example, an ABI PRISM 7700 Sequence Detector using 50 μl reactions consisting of 10 μl total RNA, 100 nM forward primer, 900 nM reverse primer, 100 nM probe, 1X TaqMan PCR reaction buffer (PE-Applied Biosystems), 5.5 mM $MgCl_2$, 300 μM each dATP, dCTP, dGTP, and dTTP, 10U RNase Inhibitor (Promega), 1.25U AmpliTaq Gold (PE-Applied Biosystems) and 10U M-MLV Reverse Transcriptase (Promega). The thermal cycling conditions can consist of 30 min at 48°C, 10 min at 95°C, followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. Quantitation of mRNA levels is determined relative to standards generated from serially diluted total cellular RNA (300, 100, 33, 11 ng/rxn) and normalizing to ß-actin or GAPDH mRNA in

parallel TaqMan reactions. For each gene of interest an upper and lower primer and a fluorescently labeled probe are designed. Real time incorporation of SYBR Green I dye into a specific PCR product can be measured in glass capillary tubes using a lightcyler. A standard curve is generated for each primer pair using control cRNA. Values are represented as relative expression to GAPDH in each sample.

Western blotting

Nuclear extracts can be prepared using a standard micro preparation technique (see for example Andrews and Faller, 1991, *Nucleic Acids Research,* 19, 2499). Protein extracts from supernatants are prepared, for example using TCA precipitation. An equal volume of 20% TCA is added to the cell supernatant, incubated on ice for 1 hour and pelleted by centrifugation for 5 minutes. Pellets are washed in acetone, dried and resuspended in water. Cellular protein extracts are run on a 10% Bis-Tris NuPage (nuclear extracts) or 4-12% Tris-Glycine (supernatant extracts) polyacrylamide gel and transferred onto nitro-cellulose membranes. Non-specific binding can be blocked by incubation, for example, with 5% non-fat milk for 1 hour followed by primary antibody for 16 hour at 4°C. Following washes, the secondary antibody is applied, for example (1:10,000 dilution) for 1 hour at room temperature and the signal detected with SuperSignal reagent (Pierce).

Example 8: Animal Models useful to evaluate the down-regulation of VEGF and/or VEGFr gene expression

There are several animal models in which the anti-angiogenesis effect of nucleic acids of the present invention, such as siRNA, directed against VEGF, VEGFr1, VEGFr2 and/or VEGFr3 mRNAs can be tested. Typically a corneal model has been used to study angiogenesis in rat and rabbit since recruitment of vessels can easily be followed in this normally avascular tissue (Pandey *et al.,* 1995 *Science* 268: 567-569). In these models, a small Teflon or Hydron disk pretreated with an angiogenesis factor (e.g. bFGF or VEGF) is inserted into a pocket surgically created in the cornea. Angiogenesis is monitored 3 to 5 days later. siRNA directed against VEGF, VEGFr1, VEGFr2 and/or VEGFr3 mRNAs are delivered in the disk as well, or dropwise to the eye over the time course of the experiment.

In another eye model, hypoxia has been shown to cause both increased expression of VEGF and neovascularization in the retina (Pierce *et al.,* 1995 *Proc. Natl. Acad. Sci.* USA. 92: 905-909; Shweiki *et al.,* 1992 *J. Clin. Invest.* 91: 2235-2243).

In human glioblastomas, it has been shown that VEGF is at least partially responsible for tumor angiogenesis (Plate *et al.,* 1992 *Nature* 359, 845). Animal models have been developed in which glioblastoma cells are implanted subcutaneously into nude mice and the progress of tumor growth and angiogenesism is studied (Kim *et al.,* 1993 *supra;* Millauer *et al.,* 1994 *supra*).

Another animal model that addresses neovascularization involves Matrigel, an extract of basement membrane that becomes a solid gel when injected subcutaneously (Passaniti *et al.,* 1992 *Lab. Invest.* 67: 519-528). When the Matrigel is supplemented with angiogenesis factors such as VEGF, vessels grow into the Matrigel over a period of 3 to 5 days and angiogenesis can be assessed. Again, nucleic acids directed against VEGFr mRNAs are delivered in the Matrigel.

Several animal models exist for screening of anti-angiogenic agents. These include corneal vessel formation following corneal injury (Burger *et al.,* 1985 *Cornea* 4: 35-41; Lepri, *et al.,* 1994 *J. Ocular Pharmacol.* 10: 273-280; Ormerod *et al.,* 1990 *Am. J. Pathol.* 137: 1243-1252) or intracorneal growth factor implant (Grant *et al.,* 1993 *Diabetologia* 36: 282-291; Pandey *et al.* 1995 *supra;* Zieche *et al.,* 1992 *Lab. Invest.* 67: 711-715), vessel growth into Matrigel matrix containing growth factors (Passaniti *et al.,* 1992 *supra*), female reproductive organ neovascularization following hormonal manipulation (Shweiki *et al.,* 1993 *Clin. Invest.* 91: 2235-2243), several models involving inhibition of tumor growth in highly vascularized solid tumors (O'Reilly *et al.,* 1994 *Cell* 79: 315-328; Senger *et al.,* 1993 *Cancer and Metas. Rev.* 12: 303-324; Takahasi *et al.,* 1994 *Cancer Res.* 54: 4233-4237; Kim *et al.,* 1993 *supra*), and transient hypoxia-induced neovascularization in the mouse retina (Pierce *et al.,* 1995 *Proc. Natl. Acad. Sci.* USA. 92: 905-909).

The cornea model, described in Pandey et al. *supra*, is the most common and well characterized model for screening anti-angiogenic agent efficacy. This model involves an

avascular tissue into which vessels are recruited by a stimulating agent (growth factor, thermal or alkalai burn, endotoxin). The corneal model utilizes the intrastromal corneal implantation of a Teflon pellet soaked in a VEGF-Hydron solution to recruit blood vessels toward the pellet, which can be quantitated using standard microscopic and image analysis techniques. To evaluate their anti-angiogenic efficacy, nucleic acids are applied topically to the eye or bound within Hydron on the Teflon pellet itself. This avascular cornea as well as the Matrigel (see below) provide for low background assays. While the corneal model has been performed extensively in the rabbit, studies in the rat have also been conducted.

The mouse model (Passaniti et al., *supra*) is a non-tissue model that utilizes Matrigel, an extract of basement membrane (Kleinman et al., 1986) or Millipore® filter disk, which can be impregnated with growth factors and anti-angiogenic agents in a liquid form prior to injection. Upon subcutaneous administration at body temperature, the Matrigel or Millipore® filter disk forms a solid implant. VEGF embedded in the Matrigel or Millipore® filter disk is used to recruit vessels within the matrix of the Matrigel or Millipore® filter disk which can be processed histologically for endothelial cell specific vWF (factor VIII antigen) immunohistochemistry, Trichrome-Masson stain, or hemoglobin content. Like the cornea, the Matrigel or Millipore® filter disk is avascular; however, it is not tissue. In the Matrigel or Millipore® filter disk model, nucleic acids are administered within the matrix of the Matrigel or Millipore® filter disk to test their anti-angiogenic efficacy. Thus, delivery issues in this model, as with delivery of nucleic acids by Hydron-coated Teflon pellets in the rat cornea model, may be less problematic due to the homogeneous presence of the nucleic acid within the respective matrix.

Other model systems to study tumor angiogenesis is reviewed by Folkman, 1985 *Adv. Cancer. Res.*. 43, 175.

Use of murine models

For a typical systemic study involving 10 mice (20 g each) per dose group, 5 doses (1, 3, 10, 30 and 100 mg/kg daily over 14 days continuous administration), approximately 400

mg of siRNA, formulated in saline is used. A similar study in young adult rats (200 g) requires over 4 g. Parallel pharmacokinetic studies involve the use of similar quantities of siRNA further justifying the use of murine models.

Lewis lung carcinoma and B-16 melanoma murine models

Identifying a common animal model for systemic efficacy testing of nucleic acids is an efficient way of screening siRNA for systemic efficacy.

The Lewis lung carcinoma and B-16 murine melanoma models are well accepted models of primary and metastatic cancer and are used for initial screening of anti-cancer agents. These murine models are not dependent upon the use of immunodeficient mice, are relatively inexpensive, and minimize housing concerns. Both the Lewis lung and B-16 melanoma models involve subcutaneous implantation of approximately 10^6 tumor cells from metastatically aggressive tumor cell lines (Lewis lung lines 3LL or D122, LLc-LN7; B-16-BL6 melanoma) in C57BL/6J mice. Alternatively, the Lewis lung model can be produced by the surgical implantation of tumor spheres (approximately 0.8 mm in diameter). Metastasis also can be modeled by injecting the tumor cells directly intravenously. In the Lewis lung model, microscopic metastases can be observed approximately 14 days following implantation with quantifiable macroscopic metastatic tumors developing within 21-25 days. The B-16 melanoma exhibits a similar time course with tumor neovascularization beginning 4 days following implantation. Since both primary and metastatic tumors exist in these models after 21-25 days in the same animal, multiple measurements can be taken as indices of efficacy. Primary tumor volume and growth latency as well as the number of micro- and macroscopic metastatic lung foci or number of animals exhibiting metastases can be quantitated. The percent increase in lifespan can also be measured. Thus, these models provide suitable primary efficacy assays for screening systemically administered siRNA nucleic acids and siRNA nucleic acid formulations .

In the Lewis lung and B-16 melanoma models, systemic pharmacotherapy with a wide variety of agents usually begins 1-7 days following tumor implantation/inoculation with either continuous or multiple administration regimens. Concurrent pharmacokinetic studies

can be performed to determine whether sufficient tissue levels of siRNA can be achieved for pharmacodynamic effect to be expected. Furthermore, primary tumors and secondary lung metastases can be removed and subjected to a variety of *in vitro* studies (*i.e.* target RNA reduction).

In addition, animal models are useful in screening compounds, eg. siRNA molecules, for efficacy in treating renal failure, such as a result of autosomal dominant polycystic kidney disease (ADPKD). The Han:SPRD rat model, mice with a targeted mutation in the Pkd2 gene and congenital polycystic kidney (cpk) mice, closely resemble human ADPKD and provide animal models to evaluate the therapeutic effect of siRNA constructs that have the potential to interfere with one or more of the pathogenic elements of ADPKD mediated renal failure, such as angiogenesis. Angiogenesis may be necessary in the progression of ADPKD for growth of cyst cells as well as increased vascular permeability promoting fluid secretion into cysts. Proliferation of cystic epithelium is also a feature of ADPKD because cyst cells in culture produce soluble vascular endothelial growth factor (VEGF). VEGFr1 has also been detected in epithelial cells of cystic tubules but not in endothelial cells in the vasculature of cystic kidneys or normal kidneys. VEGFr2 expression is increased in endothelial cells of cyst vessels and in endothelial cells during renal ischemia-reperfusion. It is proposed that inhibition of VEGF receptors with anti-VEGFr1 and anti-VEGFr2 siRNA molecules would attenuate cyst formation, renal failure and mortality in ADPKD. Anti-VEGFr2 siRNA molecules would therefore be designed to inhibit angiogenesis involved in cyst formation. As VEGFr1 is present in cystic epithelium and not in vascular endothelium of cysts, it is proposed that anti-VEGFr1 siRNA molecules would attenuate cystic epithelial cell proliferation and apoptosis which would in turn lead to less cyst formation. Further, it is proposed that VEGF produced by cystic epithelial cells is one of the stimuli for angiogenesis as well as epithelial cell proliferation and apoptosis. The use of Han:SPRD rats (see for eaxmple Kaspareit-Rittinghausen *et al.,* 1991, *Am.J.Pathol.* 139, 693-696), mice with a targeted mutation in the Pkd2 gene (Pkd2-/- mice, see for example Wu *et al.,* 2000, *Nat.Genet.* 24, 75-78) and cpk mice (see for example Woo *et al.,* 1994, *Nature,* 368, 750-753) all provide animal models to study the efficacy of siRNA molecles of the invention against VEGFr1 and VEGFr2 mediated renal failure.

VEGF, VEGFr1 VGFR2 and/or VEGFr3 protein levels can be measured clinically or experimentally by FACS analysis. VEGF, VEGFr1 VGFR2 and/or VEGFr3 encoded mRNA levels are assessed by Northern analysis, RNase-protection, primer extension analysis and/or quantitative RT-PCR. siRNA nucleic acids that block VEGF, VEGFr1 VGFR2 and/or VEGFr3 protein encoding mRNAs and therefore result in decreased levels of VEGF, VEGFr1 VGFR2 and/or VEGFr3 activity by more than 20% *in vitro* can be identified.

Example 9: siNA-mediated inhibition of angiogenesis *in vivo*

The purpose of this study was to assess the anti-angiogenic activity of siNA targeted against VEGFr1 in the rat cornea model of VEGF induced angiogenesis (see above). The siNA molecules have matched inverted controls, which are inactive since they are not able to interact with the RNA target. The siNA molecules and VEGF were co-delivered using the filter disk method: Nitrocellulose filter disks (Millipore®) of 0.057 diameter were immersed in appropriate solutions and were surgically implanted in rat cornea as described by Pandey *et al., supra*.

The stimulus for angiogenesis in this study was the treatment of the filter disk with 30 μM VEGF, which is implanted within the cornea's stroma. This dose yields reproducible neovascularization stemming from the pericorneal vascular plexus growing toward the disk in a dose-response study 5 days following implant. Filter disks treated only with the vehicle for VEGF show no angiogenic response. The siNA were co-adminstered with VEGF on a disk in two different siNA concentrations. One concern with the simultaneous administration is that the siNA would not be able to inhibit angiogenesis since VEGF receptors could be stimulated. However, Applicant has observed that in low VEGF doses, the neovascular response reverts to normal, suggesting that the VEGF stimulus is essential for maintaining the angiogenic response. Blocking the production of VEGF receptors using simultaneous administration of anti-VEGF-R mRNA siNA could attenuate the normal neovascularization induced by the filter disk treated with VEGF.

Materials and Methods:

Test Compounds and Controls

R&D Systems VEGF, carrier free at 75 μM in 82 mM Tris-Cl, pH 6.9

siNA, 1.67 μG/μL, SITE 2340 (SEQ ID NO: 2; SEQ ID NO: 6) sense/antisense

siNA, 1.67 μG/μL, INVERTED CONTROL FOR SITE 2340 (SEQ ID NO: 19; SEQ ID NO: 20) sense/antisense

siNA 1.67 μg/μL, Site 2340 (SEQ ID NO: 419; SEQ ID NO: 420) sense/antisense

Animals

Harlan Sprague-Dawley Rats, Approximately 225-250g

45 males, 5 animals per group.

Husbandry

Animals are housed in groups of two. Feed, water, temperature and humidity are determined according to Pharmacology Testing Facility performance standards (SOP's) which are in accordance with the 1996 Guide for the Care and Use of Laboratory Animals (NRC). Animals are acclimated to the facility for at least 7 days prior to experimentation. During this time, animals are observed for overall health and sentinels are bled for baseline serology.

Experimental Groups

Each solution (VEGF and siNAs) was prepared as a **1X** solution for final concentrations shown in the experimental groups described in **Table III**.

siNA Annealing Conditions

siNA sense and antisense strands are annealed for 1 minute in H_2O at 1.67mg/mL/strand followed by a 1 hour incubation at 37°C producing 3.34 mg/mL of duplexed siNA. For the 20μg/eye treatment, 6 μLs of the 3.34 mg/mL duplex is injected into the eye (see below). The 3.34 mg/mL duplex siNA can then be serially diluted for dose response assays.

Preparation of VEGF Filter Disk

For corneal implantation, 0.57 mm diameter nitrocellulose disks, prepared from 0.45 μm pore diameter nitrocellulose filter membranes (Millipore Corporation), were soaked for 30 min in 1 μL of 75 μM VEGF in 82 mM Tris·HCl (pH 6.9) in covered petri dishes on ice. Filter disks soaked only with the vehicle for VEGF (83 mM Tris-Cl pH 6.9) elicit no angiogenic response.

Corneal surgery

The rat corneal model used in this study was a modified from Koch *et al. Supra* and Pandey *et al., supra*. Briefly, corneas were irrigated with 0.5% povidone iodine solution followed by normal saline and two drops of 2% lidocaine. Under a dissecting microscope (Leica MZ-6), a stromal pocket was created and a presoaked filter disk (see above) was inserted into the pocket such that its edge was 1 mm from the corneal limbus.

Intraconjunctival injection of test solutions

Immediately after disk insertion, the tip of a 40-50 μm OD injector (constructed in our laboratory) was inserted within the conjunctival tissue 1 mm away from the edge of the corneal limbus that was directly adjacent to the VEGF-soaked filter disk. Six hundred nanoliters of test solution (siNA, inverted control or sterile water vehicle) were dispensed at a rate of 1.2 μL/min using a syringe pump (Kd Scientific). The injector was then removed, serially rinsed in 70% ethanol and sterile water and immersed in sterile water between each injection. Once the test solution was injected, closure of the eyelid was maintained using

microaneurism clips until the animal began to recover gross motor activity. Following treatment, animals were warmed on a heating pad at 37°C.

Quantitation of angiogenic response

Five days after disk implantation, animals were euthanized following administration of 0.4 mg/kg atropine and corneas were digitally imaged. The neovascular surface area (NSA, expressed in pixels) was measured *postmortem* from blood-filled corneal vessels using computerized morphometry (Image Pro Plus, Media Cybernetics, v2.0). The individual mean NSA was determined in triplicate from three regions of identical size in the area of maximal neovascularization between the filter disk and the limbus. The number of pixels corresponding to the blood-filled corneal vessels in these regions was summated to produce an index of NSA. A group mean NSA was then calculated. Data from each treatment group were normalized to VEGF/siNA vehicle-treated control NSA and finally expressed as percent inhibition of VEGF-induced angiogenesis.

Statistics

After determining the normality of treatment group means, group mean percent inhibition of VEGF-induced angiogenesis was subjected to a one-way analysis of variance. This was followed by two post-hoc tests for significance including Dunnett's (comparison to VEGF control) and Tukey-Kramer (all other group mean comparisons) at alpha = 0.05. Statistical analyses were performed using JMP v.3.1.6 (SAS Institute).

Results are graphically represented in **Figure 12.** As shown in **Figure 12,** VEGFr1 site 4229 active siNA (RPI 29695/29699) at three concentrations were effective at inhibiting angiogenesis compared to the inverted siNA control (RPI 2983/29984) and the VEGF control. A chemically modified version of the VEGFr1 site 4229 active siNA comprising a sense strand having 2'-deoxy-2'-fluoro pyrimidines and ribo purines with 5' and 3' terminal inverted deoxyabasic residues (RPI 30196) and an antisense strand having having 2'-deoxy-2'-fluoro pyrimidines and ribo purines with a terminal 3'-phosphorothioate internucleotide linkage (RPI 30416), showed similar inhibition. (Data not shown) This result shows siNA

molecules of differing chemically modified composition of the invention are capable of significantly inhibiting angiogenesis *in vivo*.

Example 10: RNAi mediated inhibition of VEGF and/or VEGFr RNA expression

siNA constructs (**Table III**) are tested for efficacy in reducing VEGF and/or VEGFr RNA expression in, for example, HUVEC, HMVEC, or A375 cells. Cells are plated approximately 24h before transfection in 96-well plates at 5,000-7,500 cells/well, 100 µl/well, such that at the time of transfection cells are 70-90% confluent. For transfection, annealed siNAs are mixed with the transfection reagent (Lipofectamine 2000, Invitrogen) in a volume of 50 µl/well and incubated for 20 min. at room temperature. The siNA transfection mixtures are added to cells to give a final siNA concentration of 25 nM in a volume of 150 µl. Each siNA transfection mixture is added to 3 wells for triplicate siNA treatments. Cells are incubated at 37° for 24h in the continued presence of the siNA transfection mixture. At 24h, RNA is prepared from each well of treated cells. The supernatants with the transfection mixtures are first removed and discarded, then the cells are lysed and RNA prepared from each well. Target gene expression following treatment is evaluated by RT-PCR for the target gene and for a control gene (36B4, an RNA polymerase subunit) for normalization. The triplicate data is averaged and the standard deviations determined for each treatment. Normalized data are graphed and the percent reduction of target mRNA by active siNAs in comparison to their respective inverted control siNAs is determined.

Figure 13 shows a non-limiting example of reduction of VEGFr1 mRNA in A375 cells mediated by chemically-modified siNAs that target VEGFr1 mRNA. A549 cells were transfected with 0.25 ug/well of lipid complexed with 25 nM siNA. A screen of siNA constructs (Stabilization "Stab" chemistries are shown in **Table IV**, constructs are referred to by RPI number, see **Table III**) comprising Stab 4/5 chemistry (RPI 31190/31193), Stab 1/2 chemistry (RPI 31183/31186 and RPI 31184/31187), and unmodified RNA (RPI 30075/30076) were compared to untreated cells, matched chemistry inverted control siNA constructs (RPI 31208/31211, RPI 31201/31204, RPI 31202/31205, and RPI 30077/30078), scrambled siNA control constructs (Scram1 and Scram2), and cells transfected with lipid

alone (transfection control). As shown in the figure, all of the siNA constructs significantly reduce VEGFr1 RNA expression. Additional stabilization chemistries as described in **Table IV** are similarly assayed for activity. These siNA constructs are compared to appropriate matched chemistry inverted controls. In addition, the siNA constructs are also compared to untreated cells, cells transfected with lipid and scrambled siNA constructs, and cells transfected with lipid alone (transfection control).

Example 11: Indications

The present body of knowledge in VEGF and/or VEGFr research indicates the need for methods to assay VEGF and/or VEGFr activity and for compounds that can regulate VEGF and/or VEGFr expression for research, diagnostic, and therapeutic use. As described herein, the nucleic acid molecules of the present invention can be used in assays to diagnose disease state related of VEGF and/or VEGFr levels. In addition, the nucleic acid molecules can be used to treat disease state related to VEGF and/or VEGFr levels.

Particular conditions and disease states that can be associated with VEGF and/or VEGFr expression modulation include, but are not limited to:

1) Tumor angiogenesis: Angiogenesis has been shown to be necessary for tumors to grow into pathological size (Folkman, 1971, *PNAS* 76, 5217-5221; Wellstein & Czubayko, 1996, *Breast Cancer Res and Treatment* 38, 109-119). In addition, it allows tumor cells to travel through the circulatory system during metastasis. Increased levels of gene expression of a number of angiogenic factors such as vascular endothelial growth factor (VEGF) have been reported in vascularized and edema-associated brain tumors (Berkman *et al.,* 1993 *J. Clini. Invest.* 91, 153). A more direct demostration of the role of VEGF in tumor angiogenesis was demonstrated by Jim Kim *et al.,* 1993 *Nature* 362,841 wherein, monoclonal antibodies against VEGF were successfully used to inhibit the growth of rhabdomyosarcoma, glioblastoma multiforme cells in nude mice. Similarly, expression of a dominant negative mutated form of the flt-1 VEGF receptor inhibits vascularization induced by human glioblastoma cells in nude mice (Millauer *et al.,* 1994, *Nature* 367, 576). Specific

tumor/cancer types that can be targeted using the nucleic acid molecules of the invention include but are not limited to the tumor/cancer types described herein.

2) Ocular diseases: Neovascularization has been shown to cause or exacerbate ocular diseases including, but not limited to, macular degeneration, neovascular glaucoma, diabetic retinopathy, myopic degeneration, and trachoma (Norrby, 1997, *APMIS* 105, 417-437). Aiello *et al.,* 1994 *New Engl. J. Med.* 331, 1480, showed that the ocular fluid of a majority of patients suffering from diabetic retinopathy and other retinal disorders contains a high concentration of VEGF. Miller *et al.*, 1994 *Am. J. Pathol.* 145, 574, reported elevated levels of VEGF mRNA in patients suffering from retinal ischemia. These observations support a direct role for VEGF in ocular diseases. Other factors, including those that stimulate VEGF synthesis, may also contribute to these indications.

3) Dermatological Disorders: Many indications have been identified which may beangiogenesis dependent, including but not limited to, psoriasis, verruca vulgaris, angiofibroma of tuberous sclerosis, pot-wine stains, Sturge Weber syndrome, Kippel-Trenaunay-Weber syndrome, and Osler-Weber-Rendu syndrome (Norrby, *supra*). Intradermal injection of the angiogenic factor b-FGF demonstrated angiogenesis in nude mice (Weckbecker et al., 1992, *Angiogenesis: Key principles-Science-Technology-Medicine*, ed R. Steiner). Detmar *et al.,* 1994 *J. Exp. Med.* 180, 1141 reported that VEGF and its receptors were over-expressed in psoriatic skin and psoriatic dermal microvessels, suggesting that VEGF plays a significant role in psoriasis.

4) Rheumatoid arthritis: Immunohistochemistry and *in situ* hybridization studies on tissues from the joints of patients suffering from rheumatoid arthritis show an increased level of VEGF and its receptors (Fava *et al.,* 1994 *J. Exp. Med.* 180, 341). Additionally, Koch *et al.,* 1994 *J. Immunol.* 152, 4149, found that VEGF-specific antibodies were able to significantly reduce the mitogenic activity of synovial tissues from patients suffering from rheumatoid arthritis. These observations support a direct role for VEGF in rheumatoid arthritis. Other angiogenic factors including those of the present invention may also be involved in arthritis.

5) Endometriosis: Various studies indicate that VEGF is directly implicated in endometriosis. In one study, VEGF concentrations measured by ELISA in peritoneal fluid were found to be significantly higher in women with endometriosis than in women without endometriosis (24.1 ± 15 ng/ml vs 13.3 ± 7.2 ng/ml in normals). In patients with endometriosis, higher concentrations of VEGF were detected in the proliferative phase of the menstrual cycle (33 ± 13 ng/ml) compared to the secretory phase (10.7 ± 5 ng/ml). The cyclic variation was not noted in fluid from normal patients (McLaren *et al.,* 1996, *Human Reprod.* 11, 220-223). In another study, women with moderate to severe endometriosis had significantly higher concentrations of peritoneal fluid VEGF than women without endometriosis. There was a positive correlation between the severity of endometriosis and the concentration of VEGF in peritoneal fluid. In human endometrial biopsies, VEGF expression increased relative to the early proliferative phase approximately 1.6-, 2-, and 3.6-fold in midproliferative, late proliferative, and secretory endometrium (Shifren *et al.,* 1996, *J. Clin. Endocrinol. Metab.* 81, 3112-3118). In a third study, VEGF-positive staining of human ectopic endometrium was shown to be localized to macrophages (double immunofluorescent staining with CD14 marker). Peritoneal fluid macrophages demonstrated VEGF staining in women with and without endometriosis. However, increased activation of macrophages (acid phosphatatse activity) was demonstrated in fluid from women with endometriosis compared with controls. Peritoneal fluid macrophage conditioned media from patients with endometriosis resulted in significantly increased cell proliferation ([^{3}H] thymidine incorporation) in HUVEC cells compared to controls. The percentage of peritoneal fluid macrophages with VEGFr2 mRNA was higher during the secretory phase, and significantly higher in fluid from women with endometriosis (80 ± 15%) compared with controls (32 ± 20%). Flt-mRNA was detected in peritoneal fluid macrophages from women with and without endometriosis, but there was no difference between the groups or any evidence of cyclic dependence (McLaren *et al.,* 1996, *J. Clin. Invest.* 98, 482-489). In the early proliferative phase of the menstrual cycle, VEGF has been found to be expressed in secretory columnar epithelium (estrogen-responsive) lining both the oviducts and the uterus in female mice. During the secretory phase, VEGF expression was shown to have shifted to the underlying stroma composing the functional endometrium. In addition to examining the endometium, neovascularization of ovarian

follicles and the corpus luteum, as well as angiogenesis in embryonic implantation sites have been analyzed. For these processes, VEGF was expressed in spatial and temporal proximity to forming vasculature (Shweiki *et al.,* 1993, *J. Clin. Invest.* 91, 2235-2243).

6) Kidney disease: Autosomal dominant polycystic kidney disease (ADPKD) is the most common life threatening hereditary disease in the USA. It affects about 1:400 to 1:1000 people and approximately 50% of people with ADPKD develop renal failure. ADPKD accounts for about 5-10% of end-stage renal failure in the USA, requiring dialysis and renal transplantation. Angiogenesis is implicated in the progression of ADPKD for growth of cyst cells, as well as increased vascular permeability promoting fluid secretion into cysts. Proliferation of cystic epithelium is a feature of ADPKD because cyst cells in culture produce soluble vascular endothelial growth factor (VEGF). VEGFr1 has been detected in epithelial cells of cystic tubules but not in endothelial cells in the vasculature of cystic kidneys or normal kidneys. VEGFr2 expression is increased in endothelial cells of cyst vessels and in endothelial cells during renal ischemia-reperfusion.

The use of radiation treatments and chemotherapeutics, such as Gemcytabine and cyclophosphamide, are non-limiting examples of chemotherapeutic agents that can be combined with or used in conjunction with the nucleic acid molecules (*e.g.* siNA molecules) of the instant invention. Those skilled in the art will recognize that other anti-cancer compounds and therapies can similarly be readily combined with the nucleic acid molecules of the instant invention (*e.g.* siNA molecules) and are hence within the scope of the instant invention. Such compounds and therapies are well known in the art (see for example *Cancer: Principles and Pranctice of Oncology*, Volumes 1 and 2, eds Devita, V.T., Hellman, S., and Rosenberg, S.A., J.B. Lippincott Company, Philadelphia, USA; incorporated herein by reference) and include, without limitation, folates, antifolates, pyrimidine analogs, fluoropyrimidines, purine analogs, adenosine analogs, topoisomerase I inhibitors, anthrapyrazoles, retinoids, antibiotics, anthacyclins, platinum analogs, alkylating agents, nitrosoureas, plant derived compounds such as vinca alkaloids, epipodophyllotoxins, tyrosine kinase inhibitors, taxols, radiation therapy, surgery, nutritional supplements, gene therapy, radiotherapy, for example 3D-CRT, immunotoxin therapy, for example ricin, and

monoclonal antibodies. Specific examples of chemotherapeutic compounds that can be combined with or used in conjuction with the nucleic acid molecules of the invention include, but are not limited to, Paclitaxel; Docetaxel; Methotrexate; Doxorubin; Edatrexate; Vinorelbine; Tomaxifen; Leucovorin; 5-fluoro uridine (5-FU); Ionotecan; Cisplatin; Carboplatin; Amsacrine; Cytarabine; Bleomycin; Mitomycin C; Dactinomycin; Mithramycin; Hexamethylmelamine; Dacarbazine; L-asperginase; Nitrogen mustard; Melphalan, Chlorambucil; Busulfan; Ifosfamide; 4-hydroperoxycyclophosphamide; Thiotepa; Irinotecan (CAMPTOSAR®, CPT-11, Camptothecin-11, Campto) Tamoxifen; Herceptin; IMC C225; ABX-EGF; and combinations thereof. The above list of compounds are non-limiting examples of compounds and/or methods that can be combined with or used in conjunction with the nucleic acid molecules (*e.g.* siNA) of the instant invention. Those skilled in the art will recognize that other drug compounds and therapies can similarly be readily combined with the nucleic acid molecules of the instant invention (e.g., siNA molecules) are hence within the scope of the instant invention.

Example 12: Diagnostic uses

The siNA molecules of the invention can be used in a variety of diagnostic applications, such as in the identification of molecular targets (e.g., RNA) in a variety of applications, for example, in clinical, industrial, environmental, agricultural and/or research settings. Such diagnostic use of siNA molecules involves utilizing reconstituted RNAi systems, for example, using cellular lysates or partially purified cellular lysates. siNA molecules of this invention can be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of endogenous or exogenous, for example viral, RNA in a cell. The close relationship between siNA activity and the structure of the target RNA allows the detection of mutations in any region of the molecule, which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple siNA molecules described in this invention, one can map nucleotide changes, which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with siNA molecules can be used to inhibit gene expression and define the role of specified gene products in the progression of disease or infection. In this manner, other genetic targets can be defined as important mediators of the disease. These experiments will

lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple siNA molecules targeted to different genes, siNA molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations siNA molecules and/or other chemical or biological molecules). Other *in vitro* uses of siNA molecules of this invention are well known in the art, and include detection of the presence of mRNAs associated with a disease, infection, or related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a siNA using standard methodologies, for example, fluorescence resonance emission transfer (FRET).

In a specific example, siNA molecules that cleave only wild-type or mutant forms of the target RNA are used for the assay. The first siNA molecules (*i.e.*, those that cleave only wild-type forms of target RNA) are used to identify wild-type RNA present in the sample and the second siNA molecules (*i.e.*, those that cleave only mutant forms of target RNA) are used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA are cleaved by both siNA molecules to demonstrate the relative siNA efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus, each analysis requires two siNA molecules, two substrates and one unknown sample, which is combined into six reactions. The presence of cleavage products is determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, disease related or infection related) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels is adequate and decreases the cost of the initial diagnosis. Higher mutant form to wild-type ratios are correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims. The present invention teaches one skilled in the art to test various combinations and/or substitutions of chemical modifications described herein toward generating nucleic acid constructs with improved activity for mediating RNAi activity. Such improved activity can comprise improved stability, improved bioavailability, and/or improved activation of cellular responses mediating RNAi. Therefore, the specific embodiments described herein are not limiting and one skilled in the art can readily appreciate that specific combinations of the modifications described herein can be tested without undue experimentation toward identifying siNA molecules with improved RNAi activity.

The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and

expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

Table I: VEGF and VEGFr Accession Numbers

NM_005429
Homo sapiens vascular endothelial growth factor C (VEGFC), mRNA
gi|19924300|ref|NM_005429.2|[19924300]

NM_003376
Homo sapiens vascular endothelial growth factor (VEGF), mRNA
gi|19923239|ref|NM_003376.2|[19923239]

AF095785
Homo sapiens vascular endothelial growth factor (VEGF) gene, promoter region and partial cds
gi|4154290|gb|AF095785.1|[4154290]

NM_003377
Homo sapiens vascular endothelial growth factor B (VEGFB), mRNA
gi|20070172|ref|NM_003377.2|[20070172]

AF486837
Homo sapiens vascular endothelial growth factor isoform VEGF165 (VEGF) mRNA, complete cds
gi|19909064|gb|AF486837.1|[19909064]

AF468110

Homo sapiens vascular endothelial growth factor B isoform (VEGFB) gene, complete cds, alternatively spliced
gi|18766397|gb|AF468110.1|[18766397]

AF437895
Homo sapiens vascular endothelial growth factor (VEGF) gene, partial cds
gi|16660685|gb|AF437895.1|AF437895[16660685]

AY047581
Homo sapiens vascular endothelial growth factor (VEGF) mRNA, complete cds
gi|15422108|gb|AY047581.1|[15422108]

AF063657
Homo sapiens vascular endothelial growth factor receptor (FLT1) mRNA, complete cds
gi|3132830|gb|AF063657.1|AF063657[3132830]

AF092127
Homo sapiens vascular endothelial growth factor (VEGF) gene, partial sequence
gi|4139168|gb|AF092127.1|AF092127[4139168]

AF092126
Homo sapiens vascular endothelial growth factor (VEGF) gene, 5' UTR
gi|4139167|gb|AF092126.1|AF092126[4139167]

AF092125
Homo sapiens vascular endothelial growth factor (VEGF) gene, partial cds
gi|4139165|gb|AF092125.1|AF092125[4139165]

E15157
Human VEGF mRNA
gi|5709840|dbj|E15157.1||pat|JP|1998052285|2[5709840]

E15156
Human VEGF mRNA
gi|5709839|dbj|E15156.1||pat|JP|1998052285|1[5709839]

E14233
Human mRNA for vascular endothelial growth factor (VEGF), complete cds
gi|5708916|dbj|E14233.1||pat|JP|1997286795|1[5708916]

AF024710
Homo sapiens vascular endothelial growth factor (VEGF) mRNA, 3'UTR
gi|2565322|gb|AF024710.1|AF024710[2565322]

AJ010438
Homo sapiens mRNA for vascular endothelial growth factor, splicing variant VEGF183
gi|3647280|emb|AJ010438.1|HSA010438[3647280]

AF098331
Homo sapiens vascular endothelial growth factor (VEGF) gene, promoter, partial sequence
gi|4235431|gb|AF098331.1|AF098331[4235431]

AF022375
Homo sapiens vascular endothelial growth factor mRNA, complete cds
gi|3719220|gb|AF022375.1|AF022375[3719220]

AH006909
vascular endothelial growth factor {alternative splicing} [human, Genomic, 414 nt 5 segments]
gi|1680143|gb|AH006909.1||bbm|191843[1680143]

U01134
Human soluble vascular endothelial cell growth factor receptor (sflt) mRNA, complete cds
gi|451321|gb|U01134.1|U01134[451321]

E14000
Human mRNA for FLT
gi|3252767|dbj|E14000.1||pat|JP|1997255700|1[3252767]

E13332
cDNA encoding vascular endodermal cell growth factor VEGF
gi|3252137|dbj|E13332.1||pat|JP|1997173075|1[3252137]

E13256
Human mRNA for FLT,complete cds
gi|3252061|dbj|E13256.1||pat|JP|1997154588|1[3252061]

AF063658
Homo sapiens vascular endothelial growth factor receptor 2 (KDR) mRNA, complete cds
gi|3132832|gb|AF063658.1|AF063658[3132832]

AJ000185
Homo Sapiens mRNA for vascular endothelial growth factor-D
gi|2879833|emb|AJ000185.1|HSAJ185[2879833]

D89630
Homo sapiens mRNA for VEGF-D, complete cds
gi|2780339|dbj|D89630.1|[2780339]

AF035121
Homo sapiens KDR/flk-1 protein mRNA, complete cds
gi|2655411|gb|AF035121.1|AF035121[2655411]

AF020393
Homo sapiens vascular endothelial growth factor C gene, partial cds and 5' upstream region
gi|2582366|gb|AF020393.1|AF020393[2582366]

Y08736
H.sapiens vegf gene, 3'UTR
gi|1619596|emb|Y08736.1|HSVEGF3UT[1619596]

X62568
H.sapiens vegf gene for vascular endothelial growth factor
gi|37658|emb|X62568.1|HSVEGF[37658]

X94216
H.sapiens mRNA for VEGF-C protein
gi|1177488|emb|X94216.1|HSVEGFC[1177488]

NM_002020
Homo sapiens fms-related tyrosine kinase 4 (FLT4), mRNA
gi|4503752|ref|NM_002020.1|[4503752]

NM_002253
Homo sapiens kinase insert domain receptor (a type III receptor tyrosine kinase) (KDR), mRNA
gi|11321596|ref|NM_002253.1|[11321596]

Table II: VEGF and VEGFr siNA and Target Sequences

VEGFR1 gi|4503748|ref|NM_002019.1

Pos	Target Sequence	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
1	GCGGACACUCCUCUCGGCU	1	1	GCGGACACUCCUCUCGGCU	1	23	AGCCGAGAGGAGUGUCCGC	428
19	UCCUCCCCGGCAGCGGCGG	2	19	UCCUCCCCGGCAGCGGCGG	2	41	CCGCCGCUGCCGGGGAGGA	429
37	GCGGCUCGGAGCGGGCUCC	3	37	GCGGCUCGGAGCGGGCUCC	3	59	GGAGCCCGCUCCGAGCCGC	430
55	CGGGGCUCGGGUGCAGCGG	4	55	CGGGGCUCGGGUGCAGCGG	4	77	CCGCUGCACCCGAGCCCCG	431
73	GCCAGCGGGCCUGGCGGCG	5	73	GCCAGCGGGCCUGGCGGCG	5	95	CGCCGCCAGGCCCGCUGGC	432
91	GAGGAUUACCCGGGGAAGU	6	91	GAGGAUUACCCGGGGAAGU	6	113	ACUUCCCCGGGUAAUCCUC	433
109	UGGUUGUCUCCUGGCUGGA	7	109	UGGUUGUCUCCUGGCUGGA	7	131	UCCAGCCAGGAGACAACCA	434
127	AGCCGCGAGACGGGCGCUC	8	127	AGCCGCGAGACGGGCGCUC	8	149	GAGCGCCCGUCUCGCGGCU	435
145	CAGGGCGCGGGGCCGGCGG	9	145	CAGGGCGCGGGGCCGGCGG	9	167	CCGCCGGCCCCGCGCCCUG	436
163	GCGGCGAACGAGAGGACGG	10	163	GCGGCGAACGAGAGGACGG	10	185	CCGUCCUCUCGUUCGCCGC	437
181	GACUCUGGCGGCCGGGUCG	11	181	GACUCUGGCGGCCGGGUCG	11	203	CGACCCGGCCGCCAGAGUC	438
199	GUUGGCCGGGGGAGCGCGG	12	199	GUUGGCCGGGGGAGCGCGG	12	221	CCGCGCUCCCCCGGCCAAC	439
217	GGCACCGGGCGAGCAGGCC	13	217	GGCACCGGGCGAGCAGGCC	13	239	GGCCUGCUCGCCCGGUGCC	440
235	CGCGUCGCGCUCACCAUGG	14	235	CGCGUCGCGCUCACCAUGG	14	257	CCAUGGUGAGCGCGACGCG	441
253	GUCAGCUACUGGGACACCG	15	253	GUCAGCUACUGGGACACCG	15	275	CGGUGUCCCAGUAGCUGAC	442
271	GGGGUCCUGCUGUGCGCGC	16	271	GGGGUCCUGCUGUGCGCGC	16	293	GCGCGCACAGCAGGACCCC	443
289	CUGCUCAGCUGUCUGCUUC	17	289	CUGCUCAGCUGUCUGCUUC	17	311	GAAGCAGACAGCUGAGCAG	444
307	CUCACAGGAUCUAGUUCAG	18	307	CUCACAGGAUCUAGUUCAG	18	329	CUGAACUAGAUCCUGUGAG	445
325	GGUUCAAAAUUAAAAGAUC	19	325	GGUUCAAAAUUAAAAGAUC	19	347	GAUCUUUUAAUUUUGAACC	446
343	CCUGAACUGAGUUUAAAAG	20	343	CCUGAACUGAGUUUAAAAG	20	365	CUUUUAAACUCAGUUCAGG	447
361	GGCACCCAGCACAUCAUGC	21	361	GGCACCCAGCACAUCAUGC	21	383	GCAUGAUGUGCUGGGUGCC	448
379	CAAGCAGGCCAGACACUGC	22	379	CAAGCAGGCCAGACACUGC	22	401	GCAGUGUCUGGCCUGCUUG	449
397	CAUCUCCAAUGCAGGGGGG	23	397	CAUCUCCAAUGCAGGGGGG	23	419	CCCCCCUGCAUUGGAGAUG	450
415	GAAGCAGCCCAUAAAUGGU	24	415	GAAGCAGCCCAUAAAUGGU	24	437	ACCAUUUAUGGGCUGCUUC	451
433	UCUUUGCCUGAAAUGGUGA	25	433	UCUUUGCCUGAAAUGGUGA	25	455	UCACCAUUUCAGGCAAAGA	452
451	AGUAAGGAAAGCGAAAGGC	26	451	AGUAAGGAAAGCGAAAGGC	26	473	GCCUUUCGCUUUCCUUACU	453
469	CUGAGCAUAACUAAAUCUG	27	469	CUGAGCAUAACUAAAUCUG	27	491	CAGAUUUAGUUAUGCUCAG	454
487	GCCUGUGGAAGAAAUGGCA	28	487	GCCUGUGGAAGAAAUGGCA	28	509	UGCCAUUUCUUCCACAGGC	455
505	AAACAAUUCUGCAGUACUU	29	505	AAACAAUUCUGCAGUACUU	29	527	AAGUACUGCAGAAUUGUUU	456
523	UUAACCUUGAACACAGCUC	30	523	UUAACCUUGAACACAGCUC	30	545	GAGCUGUGUUCAAGGUUAA	457

541	CAAGCAAACCACACUGGCU	31	541	CAAGCAAACCACACUGGCU	31	563	AGCCAGUGUGGUUUGCUUG	458
559	UUCUACAGCUGCAAAUAUC	32	559	UUCUACAGCUGCAAAUAUC	32	581	GAUAUUUGCAGCUGUAGAA	459
577	CUAGCUGUACCUACUUCAA	33	577	CUAGCUGUACCUACUUCAA	33	599	UUGAAGUAGGUACAGCUAG	460
595	AAGAAGAAGGAAACAGAAU	34	595	AAGAAGAAGGAAACAGAAU	34	617	AUUCUGUUUCCUUCUUCUU	461
613	UCUGCAAUCUAUAUAUUUA	35	613	UCUGCAAUCUAUAUAUUUA	35	635	UAAAUAUAUAGAUUGCAGA	462
631	AUUAGUGAUACAGGUAGAC	36	631	AUUAGUGAUACAGGUAGAC	36	653	GUCUACCUGUAUCACUAAU	463
649	CCUUUCGUAGAGAUGUACA	37	649	CCUUUCGUAGAGAUGUACA	37	671	UGUACAUCUCUACGAAAGG	464
667	AGUGAAAUCCCCGAAAUUA	38	667	AGUGAAAUCCCCGAAAUUA	38	689	UAAUUUCGGGGAUUUCACU	465
685	AUACACAUGACUGAAGGAA	39	685	AUACACAUGACUGAAGGAA	39	707	UUCCUUCAGUCAUGUGUAU	466
703	AGGGAGCUCGUCAUUCCCU	40	703	AGGGAGCUCGUCAUUCCCU	40	725	AGGGAAUGACGAGCUCCCU	467
721	UGCCGGGUUACGUCACCUA	41	721	UGCCGGGUUACGUCACCUA	41	743	UAGGUGACGUAACCCGGCA	468
739	AACAUCACUGUUACUUUAA	42	739	AACAUCACUGUUACUUUAA	42	761	UUAAAGUAACAGUGAUGUU	469
757	AAAAAGUUUCCACUUGACA	43	757	AAAAAGUUUCCACUUGACA	43	779	UGUCAAGUGGAAACUUUUU	470
775	ACUUUGAUCCCUGAUGGAA	44	775	ACUUUGAUCCCUGAUGGAA	44	797	UUCCAUCAGGGAUCAAAGU	471
793	AAACGCAUAAUCUGGGACA	45	793	AAACGCAUAAUCUGGGACA	45	815	UGUCCCAGAUUAUGCGUUU	472
811	AGUAGAAAGGGCUUCAUCA	46	811	AGUAGAAAGGGCUUCAUCA	46	833	UGAUGAAGCCCUUUCUACU	473
829	AUAUCAAAUGCAACGUACA	47	829	AUAUCAAAUGCAACGUACA	47	851	UGUACGUUGCAUUUGAUAU	474
847	AAAGAAAUAGGGCUUCUGA	48	847	AAAGAAAUAGGGCUUCUGA	48	869	UCAGAAGCCCUAUUUCUUU	475
865	ACCUGUGAAGCAACAGUCA	49	865	ACCUGUGAAGCAACAGUCA	49	887	UGACUGUUGCUUCACAGGU	476
883	AAUGGGCAUUUGUAUAAGA	50	883	AAUGGGCAUUUGUAUAAGA	50	905	UCUUAUACAAAUGCCCAUU	477
901	ACAAACUAUCUCACACAUC	51	901	ACAAACUAUCUCACACAUC	51	923	GAUGUGUGAGAUAGUUUGU	478
919	CGACAAACCAAUACAAUCA	52	919	CGACAAACCAAUACAAUCA	52	941	UGAUUGUAUUGGUUUGUCG	479
937	AUAGAUGUCCAAAUAAGCA	53	937	AUAGAUGUCCAAAUAAGCA	53	959	UGCUUAUUUGGACAUCUAU	480
955	ACACCACGCCCAGUCAAAU	54	955	ACACCACGCCCAGUCAAAU	54	977	AUUUGACUGGGCGUGGUGU	481
973	UUACUUAGAGGCCAUACUC	55	973	UUACUUAGAGGCCAUACUC	55	995	GAGUAUGGCCUCUAAGUAA	482
991	CUUGUCCUCAAUUGUACUG	56	991	CUUGUCCUCAAUUGUACUG	56	1013	CAGUACAAUUGAGGACAAG	483
1009	GCUACCACUCCCUUGAACA	57	1009	GCUACCACUCCCUUGAACA	57	1031	UGUUCAAGGGAGUGGUAGC	484
1027	ACGAGAGUUCAAAUGACCU	58	1027	ACGAGAGUUCAAAUGACCU	58	1049	AGGUCAUUUGAACUCUCGU	485
1045	UGGAGUUACCCUGAUGAAA	59	1045	UGGAGUUACCCUGAUGAAA	59	1067	UUUCAUCAGGGUAACUCCA	486
1063	AAAAAUAAGAGAGCUUCCG	60	1063	AAAAAUAAGAGAGCUUCCG	60	1085	CGGAAGCUCUCUUAUUUUU	487
1081	GUAAGGCGACGAAUUGACC	61	1081	GUAAGGCGACGAAUUGACC	61	1103	GGUCAAUUCGUCGCCUUAC	488
1099	CAAAGCAAUUCCCAUGCCA	62	1099	CAAAGCAAUUCCCAUGCCA	62	1121	UGGCAUGGGAAUUGCUUUG	489
1117	AACAUAUUCUACAGUGUUC	63	1117	AACAUAUUCUACAGUGUUC	63	1139	GAACACUGUAGAAUAUGUU	490
1135	CUUACUAUUGACAAAAUGC	64	1135	CUUACUAUUGACAAAAUGC	64	1157	GCAUUUUGUCAAUAGUAAG	491
1153	CAGAACAAAGACAAAGGAC	65	1153	CAGAACAAAGACAAAGGAC	65	1175	GUCCUUUGUCUUUGUUCUG	492
1171	CUUUAUACUUGUCGUGUAA	66	1171	CUUUAUACUUGUCGUGUAA	66	1193	UUACACGACAAGUAUAAAG	493

1189	AGGAGUGGACCAUCAUUCA	67	1189	AGGAGUGGACCAUCAUUCA	67	1211	UGAAUGAUGGUCCACUCCU	494
1207	AAAUCUGUUAACACCUCAG	68	1207	AAAUCUGUUAACACCUCAG	68	1229	CUGAGGUGUUAACAGAUUU	495
1225	GUGCAUAUAUAUGAUAAAG	69	1225	GUGCAUAUAUAUGAUAAAG	69	1247	CUUUAUCAUAUAUAUGCAC	496
1243	GCAUUCAUCACUGUGAAAC	70	1243	GCAUUCAUCACUGUGAAAC	70	1265	GUUUCACAGUGAUGAAUGC	497
1261	CAUCGAAAACAGCAGGUGC	71	1261	CAUCGAAAACAGCAGGUGC	71	1283	GCACCUGCUGUUUUCGAUG	498
1279	CUUGAAACCGUAGCUGGCA	72	1279	CUUGAAACCGUAGCUGGCA	72	1301	UGCCAGCUACGGUUUCAAG	499
1297	AAGCGGUCUUACCGGCUCU	73	1297	AAGCGGUCUUACCGGCUCU	73	1319	AGAGCCGGUAAGACCGCUU	500
1315	UCUAUGAAAGUGAAGGCAU	74	1315	UCUAUGAAAGUGAAGGCAU	74	1337	AUGCCUUCACUUUCAUAGA	501
1333	UUUCCCUCGCCGGAAGUUG	75	1333	UUUCCCUCGCCGGAAGUUG	75	1355	CAACUUCCGGCGAGGGAAA	502
1351	GUAUGGUUAAAAGAUGGGU	76	1351	GUAUGGUUAAAAGAUGGGU	76	1373	ACCCAUCUUUUAACCAUAC	503
1369	UUACCUGCGACUGAGAAAU	77	1369	UUACCUGCGACUGAGAAAU	77	1391	AUUUCUCAGUCGCAGGUAA	504
1387	UCUGCUCGCUAUUUGACUC	78	1387	UCUGCUCGCUAUUUGACUC	78	1409	GAGUCAAAUAGCGAGCAGA	505
1405	CGUGGCUACUCGUUAAUUA	79	1405	CGUGGCUACUCGUUAAUUA	79	1427	UAAUUAACGAGUAGCCACG	506
1423	AUCAAGGACGUAACUGAAG	80	1423	AUCAAGGACGUAACUGAAG	80	1445	CUUCAGUUACGUCCUUGAU	507
1441	GAGGAUGCAGGGAAUUAUA	81	1441	GAGGAUGCAGGGAAUUAUA	81	1463	UAUAAUUCCCUGCAUCCUC	508
1459	ACAAUCUUGCUGAGCAUAA	82	1459	ACAAUCUUGCUGAGCAUAA	82	1481	UUAUGCUCAGCAAGAUUGU	509
1477	AAACAGUCAAAUGUGUUUA	83	1477	AAACAGUCAAAUGUGUUUA	83	1499	UAAACACAUUUGACUGUUU	510
1495	AAAAACCUCACUGCCACUC	84	1495	AAAAACCUCACUGCCACUC	84	1517	GAGUGGCAGUGAGGUUUUU	511
1513	CUAAUUGUCAAUGUGAAAC	85	1513	CUAAUUGUCAAUGUGAAAC	85	1535	GUUUCACAUUGACAAUUAG	512
1531	CCCCAGAUUUACGAAAAGG	86	1531	CCCCAGAUUUACGAAAAGG	86	1553	CCUUUUCGUAAAUCUGGGG	513
1549	GCCGUGUCAUCGUUUCCAG	87	1549	GCCGUGUCAUCGUUUCCAG	87	1571	CUGGAAACGAUGACACGGC	514
1567	GACCCGGCUCUCUACCCAC	88	1567	GACCCGGCUCUCUACCCAC	88	1589	GUGGGUAGAGAGCCGGGUC	515
1585	CUGGGCAGCAGACAAAUCC	89	1585	CUGGGCAGCAGACAAAUCC	89	1607	GGAUUUGUCUGCUGCCCAG	516
1603	CUGACUUGUACCGCAUAUG	90	1603	CUGACUUGUACCGCAUAUG	90	1625	CAUAUGCGGUACAAGUCAG	517
1621	GGUAUCCCUCAACCUACAA	91	1621	GGUAUCCCUCAACCUACAA	91	1643	UUGUAGGUUGAGGGAUACC	518
1639	AUCAAGUGGUUCUGGCACC	92	1639	AUCAAGUGGUUCUGGCACC	92	1661	GGUGCCAGAACCACUUGAU	519
1657	CCCUGUAACCAUAAUCAUU	93	1657	CCCUGUAACCAUAAUCAUU	93	1679	AAUGAUUAUGGUUACAGGG	520
1675	UCCGAAGCAAGGUGUGACU	94	1675	UCCGAAGCAAGGUGUGACU	94	1697	AGUCACACCUUGCUUCGGA	521
1693	UUUUGUUCCAAUAAUGAAG	95	1693	UUUUGUUCCAAUAAUGAAG	95	1715	CUUCAUUAUUGGAACAAAA	522
1711	GAGUCCUUUAUCCUGGAUG	96	1711	GAGUCCUUUAUCCUGGAUG	96	1733	CAUCCAGGAUAAAGGACUC	523
1729	GCUGACAGCAACAUGGGAA	97	1729	GCUGACAGCAACAUGGGAA	97	1751	UUCCCAUGUUGCUGUCAGC	524
1747	AACAGAAUUGAGAGCAUCA	98	1747	AACAGAAUUGAGAGCAUCA	98	1769	UGAUGCUCUCAAUUCUGUU	525
1765	ACUCAGCGCAUGGCAAUAA	99	1765	ACUCAGCGCAUGGCAAUAA	99	1787	UUAUUGCCAUGCGCUGAGU	526
1783	AUAGAAGGAAAGAAUAAGA	100	1783	AUAGAAGGAAAGAAUAAGA	100	1805	UCUUAUUCUUUCCUUCUAU	527
1801	AUGGCUAGCACCUUGGUUG	101	1801	AUGGCUAGCACCUUGGUUG	101	1823	CAACCAAGGUGCUAGCCAU	528
1819	GUGGCUGACUCUAGAAUUU	102	1819	GUGGCUGACUCUAGAAUUU	102	1841	AAAUUCUAGAGUCAGCCAC	529

1837	UCUGGAAUCUACAUUUGCA	103	1837	UCUGGAAUCUACAUUUGCA	103	1859	UGCAAAUGUAGAUUCCAGA	530
1855	AUAGCUUCCAAUAAAGUUG	104	1855	AUAGCUUCCAAUAAAGUUG	104	1877	CAACUUUAUUGGAAGCUAU	531
1873	GGGACUGUGGGAAGAAACA	105	1873	GGGACUGUGGGAAGAAACA	105	1895	UGUUUCUUCCCACAGUCCC	532
1891	AUAAGCUUUUAUAUCACAG	106	1891	AUAAGCUUUUAUAUCACAG	106	1913	CUGUGAUAUAAAAGCUUAU	533
1909	GAUGUGCCAAAUGGGUUUC	107	1909	GAUGUGCCAAAUGGGUUUC	107	1931	GAAACCCAUUUGGCACAUC	534
1927	CAUGUUAACUUGGAAAAAA	108	1927	CAUGUUAACUUGGAAAAAA	108	1949	UUUUUUCCAAGUUAACAUG	535
1945	AUGCCGACGGAAGGAGAGG	109	1945	AUGCCGACGGAAGGAGAGG	109	1967	CCUCUCCUUCCGUCGGCAU	536
1963	GACCUGAAACUGUCUUGCA	110	1963	GACCUGAAACUGUCUUGCA	110	1985	UGCAAGACAGUUUCAGGUC	537
1981	ACAGUUAACAAGUUCUUAU	111	1981	ACAGUUAACAAGUUCUUAU	111	2003	AUAAGAACUUGUUAACUGU	538
1999	UACAGAGACGUUACUUGGA	112	1999	UACAGAGACGUUACUUGGA	112	2021	UCCAAGUAACGUCUCUGUA	539
2017	AUUUUACUGCGGACAGUUA	113	2017	AUUUUACUGCGGACAGUUA	113	2039	UAACUGUCCGCAGUAAAAU	540
2035	AAUAACAGAACAAUGCACU	114	2035	AAUAACAGAACAAUGCACU	114	2057	AGUGCAUUGUUCUGUUAUU	541
2053	UACAGUAUUAGCAAGCAAA	115	2053	UACAGUAUUAGCAAGCAAA	115	2075	UUUGCUUGCUAAUACUGUA	542
2071	AAAAUGGCCAUCACUAAGG	116	2071	AAAAUGGCCAUCACUAAGG	116	2093	CCUUAGUGAUGGCCAUUUU	543
2089	GAGCACUCCAUCACUCUUA	117	2089	GAGCACUCCAUCACUCUUA	117	2111	UAAGAGUGAUGGAGUGCUC	544
2107	AAUCUUACCAUCAUGAAUG	118	2107	AAUCUUACCAUCAUGAAUG	118	2129	CAUUCAUGAUGGUAAGAUU	545
2125	GUUUCCCUGCAAGAUUCAG	119	2125	GUUUCCCUGCAAGAUUCAG	119	2147	CUGAAUCUUGCAGGGAAAC	546
2143	GGCACCUAUGCCUGCAGAG	120	2143	GGCACCUAUGCCUGCAGAG	120	2165	CUCUGCAGGCAUAGGUGCC	547
2161	GCCAGGAAUGUAUACACAG	121	2161	GCCAGGAAUGUAUACACAG	121	2183	CUGUGUAUACAUUCCUGGC	548
2179	GGGGAAGAAAUCCUCCAGA	122	2179	GGGGAAGAAAUCCUCCAGA	122	2201	UCUGGAGGAUUUCUUCCCC	549
2197	AAGAAAGAAAUUACAAUCA	123	2197	AAGAAAGAAAUUACAAUCA	123	2219	UGAUUGUAAUUUCUUUCUU	550
2215	AGAGAUCAGGAAGCACCAU	124	2215	AGAGAUCAGGAAGCACCAU	124	2237	AUGGUGCUUCCUGAUCUCU	551
2233	UACCUCCUGCGAAACCUCA	125	2233	UACCUCCUGCGAAACCUCA	125	2255	UGAGGUUUCGCAGGAGGUA	552
2251	AGUGAUCACACAGUGGCCA	126	2251	AGUGAUCACACAGUGGCCA	126	2273	UGGCCACUGUGUGAUCACU	553
2269	AUCAGCAGUUCCACCACUU	127	2269	AUCAGCAGUUCCACCACUU	127	2291	AAGUGGUGGAACUGCUGAU	554
2287	UUAGACUGUCAUGCUAAUG	128	2287	UUAGACUGUCAUGCUAAUG	128	2309	CAUUAGCAUGACAGUCUAA	555
2305	GGUGUCCCCGAGCCUCAGA	129	2305	GGUGUCCCCGAGCCUCAGA	129	2327	UCUGAGGCUCGGGGACACC	556
2323	AUCACUUGGUUUAAAAACA	130	2323	AUCACUUGGUUUAAAAACA	130	2345	UGUUUUUAAACCAAGUGAU	557
2341	AACCACAAAAUACAACAAG	131	2341	AACCACAAAAUACAACAAG	131	2363	CUUGUUGUAUUUUGUGGUU	558
2359	GAGCCUGGAAUUAUUUUAG	132	2359	GAGCCUGGAAUUAUUUUAG	132	2381	CUAAAAUAAUUCCAGGCUC	559
2377	GGACCAGGAAGCAGCACGC	133	2377	GGACCAGGAAGCAGCACGC	133	2399	GCGUGCUGCUUCCUGGUCC	560
2395	CUGUUUAUUGAAAGAGUCA	134	2395	CUGUUUAUUGAAAGAGUCA	134	2417	UGACUCUUUCAAUAAACAG	561
2413	ACAGAAGAGGAUGAAGGUG	135	2413	ACAGAAGAGGAUGAAGGUG	135	2435	CACCUUCAUCCUCUUCUGU	562
2431	GUCUAUCACUGCAAAGCCA	136	2431	GUCUAUCACUGCAAAGCCA	136	2453	UGGCUUUGCAGUGAUAGAC	563
2449	ACCAACCAGAAGGGCUCUG	137	2449	ACCAACCAGAAGGGCUCUG	137	2471	CAGAGCCCUUCUGGUUGGU	564
2467	GUGGAAAGUUCAGCAUACC	138	2467	GUGGAAAGUUCAGCAUACC	138	2489	GGUAUGCUGAACUUUCCAC	565

2485	CUCACUGUUCAAGGAACCU	139	2485	CUCACUGUUCAAGGAACCU	139	2507	AGGUUCCUUGAACAGUGAG	566
2503	UCGGACAAGUCUAAUCUGG	140	2503	UCGGACAAGUCUAAUCUGG	140	2525	CCAGAUUAGACUUGUCCGA	567
2521	GAGCUGAUCACUCUAACAU	141	2521	GAGCUGAUCACUCUAACAU	141	2543	AUGUUAGAGUGAUCAGCUC	568
2539	UGCACCUGUGUGGCUGCGA	142	2539	UGCACCUGUGUGGCUGCGA	142	2561	UCGCAGCCACACAGGUGCA	569
2557	ACUCUCUUCUGGCUCCUAU	143	2557	ACUCUCUUCUGGCUCCUAU	143	2579	AUAGGAGCCAGAAGAGAGU	570
2575	UUAACCCUCCUUAUCCGAA	144	2575	UUAACCCUCCUUAUCCGAA	144	2597	UUCGGAUAAGGAGGGUUAA	571
2593	AAAAUGAAAAGGUCUUCUU	145	2593	AAAAUGAAAAGGUCUUCUU	145	2615	AAGAAGACCUUUUCAUUUU	572
2611	UCUGAAAUAAAGACUGACU	146	2611	UCUGAAAUAAAGACUGACU	146	2633	AGUCAGUCUUUAUUUCAGA	573
2629	UACCUAUCAAUUAUAAUGG	147	2629	UACCUAUCAAUUAUAAUGG	147	2651	CCAUUAUAAUUGAUAGGUA	574
2647	GACCCAGAUGAAGUUCCUU	148	2647	GACCCAGAUGAAGUUCCUU	148	2669	AAGGAACUUCAUCUGGGUC	575
2665	UUGGAUGAGCAGUGUGAGC	149	2665	UUGGAUGAGCAGUGUGAGC	149	2687	GCUCACACUGCUCAUCCAA	576
2683	CGGCUCCCUUAUGAUGCCA	150	2683	CGGCUCCCUUAUGAUGCCA	150	2705	UGGCAUCAUAAGGGAGCCG	577
2701	AGCAAGUGGGAGUUUGCCC	151	2701	AGCAAGUGGGAGUUUGCCC	151	2723	GGGCAAACUCCCACUUGCU	578
2719	CGGGAGAGACUUAAACUGG	152	2719	CGGGAGAGACUUAAACUGG	152	2741	CCAGUUUAAGUCUCUCCCG	579
2737	GGCAAAUCACUUGGAAGAG	153	2737	GGCAAAUCACUUGGAAGAG	153	2759	CUCUUCCAAGUGAUUUGCC	580
2755	GGGGCUUUUGGAAAAGUGG	154	2755	GGGGCUUUUGGAAAAGUGG	154	2777	CCACUUUUCCAAAAGCCCC	581
2773	GUUCAAGCAUCAGCAUUUG	155	2773	GUUCAAGCAUCAGCAUUUG	155	2795	CAAAUGCUGAUGCUUGAAC	582
2791	GGCAUUAAGAAAUCACCUA	156	2791	GGCAUUAAGAAAUCACCUA	156	2813	UAGGUGAUUUCUUAAUGCC	583
2809	ACGUGCCGGACUGUGGCUG	157	2809	ACGUGCCGGACUGUGGCUG	157	2831	CAGCCACAGUCCGGCACGU	584
2827	GUGAAAAUGCUGAAAGAGG	158	2827	GUGAAAAUGCUGAAAGAGG	158	2849	CCUCUUUCAGCAUUUUCAC	585
2845	GGGGCCACGGCCAGCGAGU	159	2845	GGGGCCACGGCCAGCGAGU	159	2867	ACUCGCUGGCCGUGGCCCC	586
2863	UACAAAGCUCUGAUGACUG	160	2863	UACAAAGCUCUGAUGACUG	160	2885	CAGUCAUCAGAGCUUUGUA	587
2881	GAGCUAAAAAUCUUGACCC	161	2881	GAGCUAAAAAUCUUGACCC	161	2903	GGGUCAAGAUUUUUAGCUC	588
2899	CACAUUGGCCACCAUCUGA	162	2899	CACAUUGGCCACCAUCUGA	162	2921	UCAGAUGGUGGCCAAUGUG	589
2917	AACGUGGUUAACCUGCUGG	163	2917	AACGUGGUUAACCUGCUGG	163	2939	CCAGCAGGUUAACCACGUU	590
2935	GGAGCCUGCACCAAGCAAG	164	2935	GGAGCCUGCACCAAGCAAG	164	2957	CUUGCUUGGUGCAGGCUCC	591
2953	GGAGGGCCUCUGAUGGUGA	165	2953	GGAGGGCCUCUGAUGGUGA	165	2975	UCACCAUCAGAGGCCCUCC	592
2971	AUUGUUGAAUACUGCAAAU	166	2971	AUUGUUGAAUACUGCAAAU	166	2993	AUUUGCAGUAUUCAACAAU	593
2989	UAUGGAAAUCUCUCCAACU	167	2989	UAUGGAAAUCUCUCCAACU	167	3011	AGUUGGAGAGAUUUCCAUA	594
3007	UACCUCAAGAGCAAACGUG	168	3007	UACCUCAAGAGCAAACGUG	168	3029	CACGUUUGCUCUUGAGGUA	595
3025	GACUUAUUUUUUCUCAACA	169	3025	GACUUAUUUUUUCUCAACA	169	3047	UGUUGAGAAAAAAUAAGUC	596
3043	AAGGAUGCAGCACUACACA	170	3043	AAGGAUGCAGCACUACACA	170	3065	UGUGUAGUGCUGCAUCCUU	597
3061	AUGGAGCCUAAGAAAGAAA	171	3061	AUGGAGCCUAAGAAAGAAA	171	3083	UUUCUUUCUUAGGCUCCAU	598
3079	AAAAUGGAGCCAGGCCUGG	172	3079	AAAAUGGAGCCAGGCCUGG	172	3101	CCAGGCCUGGCUCCAUUUU	599
3097	GAACAAGGCAAGAAACCAA	173	3097	GAACAAGGCAAGAAACCAA	173	3119	UUGGUUUCUUGCCUUGUUC	600
3115	AGACUAGAUAGCGUCACCA	174	3115	AGACUAGAUAGCGUCACCA	174	3137	UGGUGACGCUAUCUAGUCU	601

3133	AGCAGCGAAAGCUUUGCGA	175	3133	AGCAGCGAAAGCUUUGCGA	175	3155	UCGCAAAGCUUUCGCUGCU	602
3151	AGCUCCGGCUUUCAGGAAG	176	3151	AGCUCCGGCUUUCAGGAAG	176	3173	CUUCCUGAAAGCCGGAGCU	603
3169	GAUAAAAGUCUGAGUGAUG	177	3169	GAUAAAAGUCUGAGUGAUG	177	3191	CAUCACUCAGACUUUUAUC	604
3187	GUUGAGGAAGAGGAGGAUU	178	3187	GUUGAGGAAGAGGAGGAUU	178	3209	AAUCCUCCUCUUCCUCAAC	605
3205	UCUGACGGUUUCUACAAGG	179	3205	UCUGACGGUUUCUACAAGG	179	3227	CCUUGUAGAAACCGUCAGA	606
3223	GAGCCCAUCACUAUGGAAG	180	3223	GAGCCCAUCACUAUGGAAG	180	3245	CUUCCAUAGUGAUGGGCUC	607
3241	GAUCUGAUUUCUUACAGUU	181	3241	GAUCUGAUUUCUUACAGUU	181	3263	AACUGUAAGAAAUCAGAUC	608
3259	UUUCAAGUGGCCAGAGGCA	182	3259	UUUCAAGUGGCCAGAGGCA	182	3281	UGCCUCUGGCCACUUGAAA	609
3277	AUGGAGUUCCUGUCUUCCA	183	3277	AUGGAGUUCCUGUCUUCCA	183	3299	UGGAAGACAGGAACUCCAU	610
3295	AGAAAGUGCAUUCAUCGGG	184	3295	AGAAAGUGCAUUCAUCGGG	184	3317	CCCGAUGAAUGCACUUUCU	611
3313	GACCUGGCAGCGAGAAACA	185	3313	GACCUGGCAGCGAGAAACA	185	3335	UGUUUCUCGCUGCCAGGUC	612
3331	AUUCUUUUAUCUGAGAACA	186	3331	AUUCUUUUAUCUGAGAACA	186	3353	UGUUCUCAGAUAAAAGAAU	613
3349	AACGUGGUGAAGAUUUGUG	187	3349	AACGUGGUGAAGAUUUGUG	187	3371	CACAAAUCUUCACCACGUU	614
3367	GAUUUUGGCCUUGCCCGGG	188	3367	GAUUUUGGCCUUGCCCGGG	188	3389	CCCGGGCAAGGCCAAAAUC	615
3385	GAUAUUUAUAAGAACCCCG	189	3385	GAUAUUUAUAAGAACCCCG	189	3407	CGGGGUUCUUAUAAAUAUC	616
3403	GAUUAUGUGAGAAAAGGAG	190	3403	GAUUAUGUGAGAAAAGGAG	190	3425	CUCCUUUUCUCACAUAAUC	617
3421	GAUACUCGACUUCCUCUGA	191	3421	GAUACUCGACUUCCUCUGA	191	3443	UCAGAGGAAGUCGAGUAUC	618
3439	AAAUGGAUGGCUCCCGAAU	192	3439	AAAUGGAUGGCUCCCGAAU	192	3461	AUUCGGGAGCCAUCCAUUU	619
3457	UCUAUCUUUGACAAAAUCU	193	3457	UCUAUCUUUGACAAAAUCU	193	3479	AGAUUUUGUCAAAGAUAGA	620
3475	UACAGCACCAAGAGCGACG	194	3475	UACAGCACCAAGAGCGACG	194	3497	CGUCGCUCUUGGUGCUGUA	621
3493	GUGUGGUCUUACGGAGUAU	195	3493	GUGUGGUCUUACGGAGUAU	195	3515	AUACUCCGUAAGACCACAC	622
3511	UUGCUGUGGGAAAUCUUCU	196	3511	UUGCUGUGGGAAAUCUUCU	196	3533	AGAAGAUUUCCCACAGCAA	623
3529	UCCUUAGGUGGGUCUCCAU	197	3529	UCCUUAGGUGGGUCUCCAU	197	3551	AUGGAGACCCACCUAAGGA	624
3547	UACCCAGGAGUACAAAUGG	198	3547	UACCCAGGAGUACAAAUGG	198	3569	CCAUUUGUACUCCUGGGUA	625
3565	GAUGAGGACUUUUGCAGUC	199	3565	GAUGAGGACUUUUGCAGUC	199	3587	GACUGCAAAAGUCCUCAUC	626
3583	CGCCUGAGGGAAGGCAUGA	200	3583	CGCCUGAGGGAAGGCAUGA	200	3605	UCAUGCCUUCCCUCAGGCG	627
3601	AGGAUGAGAGCUCCUGAGU	201	3601	AGGAUGAGAGCUCCUGAGU	201	3623	ACUCAGGAGCUCUCAUCCU	628
3619	UACUCUACUCCUGAAAUCU	202	3619	UACUCUACUCCUGAAAUCU	202	3641	AGAUUUCAGGAGUAGAGUA	629
3637	UAUCAGAUCAUGCUGGACU	203	3637	UAUCAGAUCAUGCUGGACU	203	3659	AGUCCAGCAUGAUCUGAUA	630
3655	UGCUGGCACAGAGACCCAA	204	3655	UGCUGGCACAGAGACCCAA	204	3677	UUGGGUCUCUGUGCCAGCA	631
3673	AAAGAAAGGCCAAGAUUUG	205	3673	AAAGAAAGGCCAAGAUUUG	205	3695	CAAAUCUUGGCCUUUCUUU	632
3691	GCAGAACUUGUGGAAAAAC	206	3691	GCAGAACUUGUGGAAAAAC	206	3713	GUUUUUCCACAAGUUCUGC	633
3709	CUAGGUGAUUUGCUUCAAG	207	3709	CUAGGUGAUUUGCUUCAAG	207	3731	CUUGAAGCAAAUCACCUAG	634
3727	GCAAAUGUACAACAGGAUG	208	3727	GCAAAUGUACAACAGGAUG	208	3749	CAUCCUGUUGUACAUUUGC	635
3745	GGUAAAGACUACAUCCCAA	209	3745	GGUAAAGACUACAUCCCAA	209	3767	UUGGGAUGUAGUCUUUACC	636
3763	AUCAAUGCCAUACUGACAG	210	3763	AUCAAUGCCAUACUGACAG	210	3785	CUGUCAGUAUGGCAUUGAU	637

3781	GGAAAUAGUGGGUUUACAU	211	3781	GGAAAUAGUGGGUUUACAU	211	3803	AUGUAAACCCACUAUUUCC	638
3799	UACUCAACUCCUGCCUUCU	212	3799	UACUCAACUCCUGCCUUCU	212	3821	AGAAGGCAGGAGUUGAGUA	639
3817	UCUGAGGACUUCUUCAAGG	213	3817	UCUGAGGACUUCUUCAAGG	213	3839	CCUUGAAGAAGUCCUCAGA	640
3835	GAAAGUAUUUCAGCUCCGA	214	3835	GAAAGUAUUUCAGCUCCGA	214	3857	UCGGAGCUGAAAUACUUUC	641
3853	AAGUUUAAUUCAGGAAGCU	215	3853	AAGUUUAAUUCAGGAAGCU	215	3875	AGCUUCCUGAAUUAAACUU	642
3871	UCUGAUGAUGUCAGAUAUG	216	3871	UCUGAUGAUGUCAGAUAUG	216	3893	CAUAUCUGACAUCAUCAGA	643
3889	GUAAAUGCUUUCAAGUUCA	217	3889	GUAAAUGCUUUCAAGUUCA	217	3911	UGAACUUGAAAGCAUUUAC	644
3907	AUGAGCCUGGAAAGAAUCA	218	3907	AUGAGCCUGGAAAGAAUCA	218	3929	UGAUUCUUUCCAGGCUCAU	645
3925	AAAACCUUUGAAGAACUUU	219	3925	AAAACCUUUGAAGAACUUU	219	3947	AAAGUUCUUCAAAGGUUUU	646
3943	UUACCGAAUGCCACCUCCA	220	3943	UUACCGAAUGCCACCUCCA	220	3965	UGGAGGUGGCAUUCGGUAA	647
3961	AUGUUUGAUGACUACCAGG	221	3961	AUGUUUGAUGACUACCAGG	221	3983	CCUGGUAGUCAUCAAACAU	648
3979	GGCGACAGCAGCACUCUGU	222	3979	GGCGACAGCAGCACUCUGU	222	4001	ACAGAGUGCUGCUGUCGCC	649
3997	UUGGCCUCUCCCAUGCUGA	223	3997	UUGGCCUCUCCCAUGCUGA	223	4019	UCAGCAUGGGAGAGGCCAA	650
4015	AAGCGCUUCACCUGGACUG	224	4015	AAGCGCUUCACCUGGACUG	224	4037	CAGUCCAGGUGAAGCGCUU	651
4033	GACAGCAAACCCAAGGCCU	225	4033	GACAGCAAACCCAAGGCCU	225	4055	AGGCCUUGGGUUUGCUGUC	652
4051	UCGCUCAAGAUUGACUUGA	226	4051	UCGCUCAAGAUUGACUUGA	226	4073	UCAAGUCAAUCUUGAGCGA	653
4069	AGAGUAACCAGUAAAAGUA	227	4069	AGAGUAACCAGUAAAAGUA	227	4091	UACUUUUACUGGUUACUCU	654
4087	AAGGAGUCGGGGCUGUCUG	228	4087	AAGGAGUCGGGGCUGUCUG	228	4109	CAGACAGCCCCGACUCCUU	655
4105	GAUGUCAGCAGGCCCAGUU	229	4105	GAUGUCAGCAGGCCCAGUU	229	4127	AACUGGGCCUGCUGACAUC	656
4123	UUCUGCCAUUCCAGCUGUG	230	4123	UUCUGCCAUUCCAGCUGUG	230	4145	CACAGCUGGAAUGGCAGAA	657
4141	GGGCACGUCAGCGAAGGCA	231	4141	GGGCACGUCAGCGAAGGCA	231	4163	UGCCUUCGCUGACGUGCCC	658
4159	AAGCGCAGGUUCACCUACG	232	4159	AAGCGCAGGUUCACCUACG	232	4181	CGUAGGUGAACCUGCGCUU	659
4177	GACCACGCUGAGCUGGAAA	233	4177	GACCACGCUGAGCUGGAAA	233	4199	UUUCCAGCUCAGCGUGGUC	660
4195	AGGAAAAUCGCGUGCUGCU	234	4195	AGGAAAAUCGCGUGCUGCU	234	4217	AGCAGCACGCGAUUUUCCU	661
4213	UCCCCGCCCCCAGACUACA	235	4213	UCCCCGCCCCCAGACUACA	235	4235	UGUAGUCUGGGGGCGGGGA	662
4231	AACUCGGUGGUCCUGUACU	236	4231	AACUCGGUGGUCCUGUACU	236	4253	AGUACAGGACCACCGAGUU	663
4249	UCCACCCCACCCAUCUAGA	237	4249	UCCACCCCACCCAUCUAGA	237	4271	UCUAGAUGGGUGGGGUGGA	664
4267	AGUUUGACACGAAGCCUUA	238	4267	AGUUUGACACGAAGCCUUA	238	4289	UAAGGCUUCGUGUCAAACU	665
4285	AUUUCUAGAAGCACAUGUG	239	4285	AUUUCUAGAAGCACAUGUG	239	4307	CACAUGUGCUUCUAGAAAU	666
4303	GUAUUUAUACCCCCAGGAA	240	4303	GUAUUUAUACCCCCAGGAA	240	4325	UUCCUGGGGGUAUAAAUAC	667
4321	AACUAGCUUUUGCCAGUAU	241	4321	AACUAGCUUUUGCCAGUAU	241	4343	AUACUGGCAAAAGCUAGUU	668
4339	UUAUGCAUAUAUAAGUUUA	242	4339	UUAUGCAUAUAUAAGUUUA	242	4361	UAAACUUAUAUAUGCAUAA	669
4357	ACACCUUUAUCUUUCCAUG	243	4357	ACACCUUUAUCUUUCCAUG	243	4379	CAUGGAAAGAUAAAGGUGU	670
4375	GGGAGCCAGCUGCUUUUUG	244	4375	GGGAGCCAGCUGCUUUUUG	244	4397	CAAAAAGCAGCUGGCUCCC	671
4393	GUGAUUUUUUAAUAGUGC	245	4393	GUGAUUUUUUAAUAGUGC	245	4415	GCACUAUUAAAAAAAUCAC	672
4411	CUUUUUUUUUUUGACUAAC	246	4411	CUUUUUUUUUUUGACUAAC	246	4433	GUUAGUCAAAAAAAAAAAG	673

4429	CAAGAAUGUAACUCCAGAU	247	4429	CAAGAAUGUAACUCCAGAU	247	4451	AUCUGGAGUUACAUUCUUG	674
4447	UAGAGAAAUAGUGACAAGU	248	4447	UAGAGAAAUAGUGACAAGU	248	4469	ACUUGUCACUAUUUCUCUA	675
4465	UGAAGAACACUACUGCUAA	249	4465	UGAAGAACACUACUGCUAA	249	4487	UUAGCAGUAGUGUUCUUCA	676
4483	AAUCCUCAUGUUACUCAGU	250	4483	AAUCCUCAUGUUACUCAGU	250	4505	ACUGAGUAACAUGAGGAUU	677
4501	UGUUAGAGAAAUCCUUCCU	251	4501	UGUUAGAGAAAUCCUUCCU	251	4523	AGGAAGGAUUUCUCUAACA	678
4519	UAAACCCAAUGACUUCCCU	252	4519	UAAACCCAAUGACUUCCCU	252	4541	AGGGAAGUCAUUGGGUUUA	679
4537	UGCUCCAACCCCCGCCACC	253	4537	UGCUCCAACCCCCGCCACC	253	4559	GGUGGCGGGGGUUGGAGCA	680
4555	CUCAGGGCACGCAGGACCA	254	4555	CUCAGGGCACGCAGGACCA	254	4577	UGGUCCUGCGUGCCCUGAG	681
4573	AGUUUGAUUGAGGAGCUGC	255	4573	AGUUUGAUUGAGGAGCUGC	255	4595	GCAGCUCCUCAAUCAAACU	682
4591	CACUGAUCACCCAAUGCAU	256	4591	CACUGAUCACCCAAUGCAU	256	4613	AUGCAUUGGGUGAUCAGUG	683
4609	UCACGUACCCCACUGGGCC	257	4609	UCACGUACCCCACUGGGCC	257	4631	GGCCCAGUGGGGUACGUGA	684
4627	CAGCCCUGCAGCCCAAAAC	258	4627	CAGCCCUGCAGCCCAAAAC	258	4649	GUUUUGGGCUGCAGGGCUG	685
4645	CCCAGGGCAACAAGCCCGU	259	4645	CCCAGGGCAACAAGCCCGU	259	4667	ACGGGCUUGUUGCCCUGGG	686
4663	UUAGCCCCAGGGGAUCACU	260	4663	UUAGCCCCAGGGGAUCACU	260	4685	AGUGAUCCCCUGGGGCUAA	687
4681	UGGCUGGCCUGAGCAACAU	261	4681	UGGCUGGCCUGAGCAACAU	261	4703	AUGUUGCUCAGGCCAGCCA	688
4699	UCUCGGGAGUCCUCUAGCA	262	4699	UCUCGGGAGUCCUCUAGCA	262	4721	UGCUAGAGGACUCCCGAGA	689
4717	AGGCCUAAGACAUGUGAGG	263	4717	AGGCCUAAGACAUGUGAGG	263	4739	CCUCACAUGUCUUAGGCCU	690
4735	GAGGAAAAGGAAAAAAAGC	264	4735	GAGGAAAAGGAAAAAAAGC	264	4757	GCUUUUUUUCCUUUUCCUC	691
4753	CAAAAAGCAAGGGAGAAAA	265	4753	CAAAAAGCAAGGGAGAAAA	265	4775	UUUUCUCCCUUGCUUUUUG	692
4771	AGAGAAACCGGGAGAAGGC	266	4771	AGAGAAACCGGGAGAAGGC	266	4793	GCCUUCUCCCGGUUUCUCU	693
4789	CAUGAGAAAGAAUUUGAGA	267	4789	CAUGAGAAAGAAUUUGAGA	267	4811	UCUCAAAUUCUUUCUCAUG	694
4807	ACGCACCAUGUGGGCACGG	268	4807	ACGCACCAUGUGGGCACGG	268	4829	CCGUGCCCACAUGGUGCGU	695
4825	GAGGGGGACGGGGCUCAGC	269	4825	GAGGGGGACGGGGCUCAGC	269	4847	GCUGAGCCCCGUCCCCCUC	696
4843	CAAUGCCAUUUCAGUGGCU	270	4843	CAAUGCCAUUUCAGUGGCU	270	4865	AGCCACUGAAAUGGCAUUG	697
4861	UUCCCAGCUCUGACCCUUC	271	4861	UUCCCAGCUCUGACCCUUC	271	4883	GAAGGGUCAGAGCUGGGAA	698
4879	CUACAUUUGAGGGCCCAGC	272	4879	CUACAUUUGAGGGCCCAGC	272	4901	GCUGGGCCCUCAAAUGUAG	699
4897	CCAGGAGCAGAUGGACAGC	273	4897	CCAGGAGCAGAUGGACAGC	273	4919	GCUGUCCAUCUGCUCCUGG	700
4915	CGAUGAGGGGACAUUUUCU	274	4915	CGAUGAGGGGACAUUUUCU	274	4937	AGAAAAUGUCCCCUCAUCG	701
4933	UGGAUUCUGGGAGGCAAGA	275	4933	UGGAUUCUGGGAGGCAAGA	275	4955	UCUUGCCUCCCAGAAUCCA	702
4951	AAAAGGACAAAUAUCUUUU	276	4951	AAAAGGACAAAUAUCUUUU	276	4973	AAAAGAUAUUUGUCCUUUU	703
4969	UUUGGAACUAAAGCAAAUU	277	4969	UUUGGAACUAAAGCAAAUU	277	4991	AAUUUGCUUUAGUUCCAAA	704
4987	UUUAGACCUUUACCUAUGG	278	4987	UUUAGACCUUUACCUAUGG	278	5009	CCAUAGGUAAAGGUCUAAA	705
5005	GAAGUGGUUCUAUGUCCAU	279	5005	GAAGUGGUUCUAUGUCCAU	279	5027	AUGGACAUAGAACCACUUC	706
5023	UUCUCAUUCGUGGCAUGUU	280	5023	UUCUCAUUCGUGGCAUGUU	280	5045	AACAUGCCACGAAUGAGAA	707
5041	UUUGAUUUGUAGCACUGAG	281	5041	UUUGAUUUGUAGCACUGAG	281	5063	CUCAGUGCUACAAAUCAAA	708
5059	GGGUGGCACUCAACUCUGA	282	5059	GGGUGGCACUCAACUCUGA	282	5081	UCAGAGUUGAGUGCCACCC	709

5077	AGCCCAUACUUUUGGCUCC	283	5077	AGCCCAUACUUUUGGCUCC	283	5099	GGAGCCAAAAGUAUGGGCU	710
5095	CUCUAGUAAGAUGCACUGA	284	5095	CUCUAGUAAGAUGCACUGA	284	5117	UCAGUGCAUCUUACUAGAG	711
5113	AAAACUUAGCCAGAGUUAG	285	5113	AAAACUUAGCCAGAGUUAG	285	5135	CUAACUCUGGCUAAGUUUU	712
5131	GGUUGUCUCCAGGCCAUGA	286	5131	GGUUGUCUCCAGGCCAUGA	286	5153	UCAUGGCCUGGAGACAACC	713
5149	AUGGCCUUACACUGAAAAU	287	5149	AUGGCCUUACACUGAAAAU	287	5171	AUUUUCAGUGUAAGGCCAU	714
5167	UGUCACAUUCUAUUUUGGG	288	5167	UGUCACAUUCUAUUUUGGG	288	5189	CCCAAAAUAGAAUGUGACA	715
5185	GUAUUAAUAUAUAGUCCAG	289	5185	GUAUUAAUAUAUAGUCCAG	289	5207	CUGGACUAUAUAUUAAUAC	716
5203	GACACUUAACUCAAUUUCU	290	5203	GACACUUAACUCAAUUUCU	290	5225	AGAAAUUGAGUUAAGUGUC	717
5221	UUGGUAUUAUUCUGUUUUG	291	5221	UUGGUAUUAUUCUGUUUUG	291	5243	CAAAACAGAAUAAUACCAA	718
5239	GCACAGUUAGUUGUGAAAG	292	5239	GCACAGUUAGUUGUGAAAG	292	5261	CUUUCACAACUAACUGUGC	719
5257	GAAAGCUGAGAAGAAUGAA	293	5257	GAAAGCUGAGAAGAAUGAA	293	5279	UUCAUUCUUCUCAGCUUUC	720
5275	AAAUGCAGUCCUGAGGAGA	294	5275	AAAUGCAGUCCUGAGGAGA	294	5297	UCUCCUCAGGACUGCAUUU	721
5293	AGUUUUCUCCAUAUCAAAA	295	5293	AGUUUUCUCCAUAUCAAAA	295	5315	UUUUGAUAUGGAGAAAACU	722
5311	ACGAGGGCUGAUGGAGGAA	296	5311	ACGAGGGCUGAUGGAGGAA	296	5333	UUCCUCCAUCAGCCCUCGU	723
5329	AAAAGGUCAAUAAGGUCAA	297	5329	AAAAGGUCAAUAAGGUCAA	297	5351	UUGACCUUAUUGACCUUUU	724
5347	AGGGAAGACCCCGUCUCUA	298	5347	AGGGAAGACCCCGUCUCUA	298	5369	UAGAGACGGGGUCUUCCCU	725
5365	AUACCAACCAAACCAAUUC	299	5365	AUACCAACCAAACCAAUUC	299	5387	GAAUUGGUUUGGUUGGUAU	726
5383	CACCAACACAGUUGGGACC	300	5383	CACCAACACAGUUGGGACC	300	5405	GGUCCCAACUGUGUUGGUG	727
5401	CCAAAACACAGGAAGUCAG	301	5401	CCAAAACACAGGAAGUCAG	301	5423	CUGACUUCCUGUGUUUUGG	728
5419	GUCACGUUUCCUUUUCAUU	302	5419	GUCACGUUUCCUUUUCAUU	302	5441	AAUGAAAAGGAAACGUGAC	729
5437	UUAAUGGGGAUUCCACUAU	303	5437	UUAAUGGGGAUUCCACUAU	303	5459	AUAGUGGAAUCCCCAUUAA	730
5455	UCUCACACUAAUCUGAAAG	304	5455	UCUCACACUAAUCUGAAAG	304	5477	CUUUCAGAUUAGUGUGAGA	731
5473	GGAUGUGGAAGAGCAUUAG	305	5473	GGAUGUGGAAGAGCAUUAG	305	5495	CUAAUGCUCUUCCACAUCC	732
5491	GCUGGCGCAUAUUAAGCAC	306	5491	GCUGGCGCAUAUUAAGCAC	306	5513	GUGCUUAAUAUGCGCCAGC	733
5509	CUUUAAGCUCCUUGAGUAA	307	5509	CUUUAAGCUCCUUGAGUAA	307	5531	UUACUCAAGGAGCUUAAAG	734
5527	AAAAGGUGGUAUGUAAUUU	308	5527	AAAAGGUGGUAUGUAAUUU	308	5549	AAAUUACAUACCACCUUUU	735
5545	UAUGCAAGGUAUUUCUCCA	309	5545	UAUGCAAGGUAUUUCUCCA	309	5567	UGGAGAAAUACCUUGCAUA	736
5563	AGUUGGGACUCAGGAUAUU	310	5563	AGUUGGGACUCAGGAUAUU	310	5585	AAUAUCCUGAGUCCCAACU	737
5581	UAGUUAAUGAGCCAUCACU	311	5581	UAGUUAAUGAGCCAUCACU	311	5603	AGUGAUGGCUCAUUAACUA	738
5599	UAGAAGAAAAGCCCAUUUU	312	5599	UAGAAGAAAAGCCCAUUUU	312	5621	AAAAUGGGCUUUUCUUCUA	739
5617	UCAACUGCUUUGAAACUUG	313	5617	UCAACUGCUUUGAAACUUG	313	5639	CAAGUUUCAAAGCAGUUGA	740
5635	GCCUGGGGUCUGAGCAUGA	314	5635	GCCUGGGGUCUGAGCAUGA	314	5657	UCAUGCUCAGACCCCAGGC	741
5653	AUGGGAAUAGGGAGACAGG	315	5653	AUGGGAAUAGGGAGACAGG	315	5675	CCUGUCUCCCUAUUCCCAU	742
5671	GGUAGGAAAGGGCGCCUAC	316	5671	GGUAGGAAAGGGCGCCUAC	316	5693	GUAGGCGCCCUUUCCUACC	743
5689	CUCUUCAGGGUCUAAAGAU	317	5689	CUCUUCAGGGUCUAAAGAU	317	5711	AUCUUUAGACCCUGAAGAG	744
5707	UCAAGUGGGCCUUGGAUCG	318	5707	UCAAGUGGGCCUUGGAUCG	318	5729	CGAUCCAAGGCCCACUUGA	745

5725	GCUAAGCUGGCUCUGUUUG	319	5725	GCUAAGCUGGCUCUGUUUG	319	5747	CAAACAGAGCCAGCUUAGC	746
5743	GAUGCUAUUUAUGCAAGUU	320	5743	GAUGCUAUUUAUGCAAGUU	320	5765	AACUUGCAUAAAUAGCAUC	747
5761	UAGGGUCUAUGUAUUUAGG	321	5761	UAGGGUCUAUGUAUUUAGG	321	5783	CCUAAAUACAUAGACCCUA	748
5779	GAUGCGCCUACUCUUCAGG	322	5779	GAUGCGCCUACUCUUCAGG	322	5801	CCUGAAGAGUAGGCGCAUC	749
5797	GGUCUAAAGAUCAAGUGGG	323	5797	GGUCUAAAGAUCAAGUGGG	323	5819	CCCACUUGAUCUUUAGACC	750
5815	GCCUUGGAUCGCUAAGCUG	324	5815	GCCUUGGAUCGCUAAGCUG	324	5837	CAGCUUAGCGAUCCAAGGC	751
5833	GGCUCUGUUUGAUGCUAUU	325	5833	GGCUCUGUUUGAUGCUAUU	325	5855	AAUAGCAUCAAACAGAGCC	752
5851	UUAUGCAAGUUAGGGUCUA	326	5851	UUAUGCAAGUUAGGGUCUA	326	5873	UAGACCCUAACUUGCAUAA	753
5869	AUGUAUUUAGGAUGUCUGC	327	5869	AUGUAUUUAGGAUGUCUGC	327	5891	GCAGACAUCCUAAAUACAU	754
5887	CACCUUCUGCAGCCAGUCA	328	5887	CACCUUCUGCAGCCAGUCA	328	5909	UGACUGGCUGCAGAAGGUG	755
5905	AGAAGCUGGAGAGGCAACA	329	5905	AGAAGCUGGAGAGGCAACA	329	5927	UGUUGCCUCUCCAGCUUCU	756
5923	AGUGGAUUGCUGCUUCUUG	330	5923	AGUGGAUUGCUGCUUCUUG	330	5945	CAAGAAGCAGCAAUCCACU	757
5941	GGGGAGAAGAGUAUGCUUC	331	5941	GGGGAGAAGAGUAUGCUUC	331	5963	GAAGCAUACUCUUCUCCCC	758
5959	CCUUUUAUCCAUGUAAUUU	332	5959	CCUUUUAUCCAUGUAAUUU	332	5981	AAAUUACAUGGAUAAAAGG	759
5977	UAACUGUAGAACCUGAGCU	333	5977	UAACUGUAGAACCUGAGCU	333	5999	AGCUCAGGUUCUACAGUUA	760
5995	UCUAAGUAACCGAAGAAUG	334	5995	UCUAAGUAACCGAAGAAUG	334	6017	CAUUCUUCGGUUACUUAGA	761
6013	GUAUGCCUCUGUUCUUAUG	335	6013	GUAUGCCUCUGUUCUUAUG	335	6035	CAUAAGAACAGAGGCAUAC	762
6031	GUGCCACAUCCUUGUUUAA	336	6031	GUGCCACAUCCUUGUUUAA	336	6053	UUAAACAAGGAUGUGGCAC	763
6049	AAGGCUCUCUGUAUGAAGA	337	6049	AAGGCUCUCUGUAUGAAGA	337	6071	UCUUCAUACAGAGAGCCUU	764
6067	AGAUGGGACCGUCAUCAGC	338	6067	AGAUGGGACCGUCAUCAGC	338	6089	GCUGAUGACGGUCCCAUCU	765
6085	CACAUUCCCUAGUGAGCCU	339	6085	CACAUUCCCUAGUGAGCCU	339	6107	AGGCUCACUAGGGAAUGUG	766
6103	UACUGGCUCCUGGCAGCGG	340	6103	UACUGGCUCCUGGCAGCGG	340	6125	CCGCUGCCAGGAGCCAGUA	767
6121	GCUUUUGUGGAAGACUCAC	341	6121	GCUUUUGUGGAAGACUCAC	341	6143	GUGAGUCUUCCACAAAAGC	768
6139	CUAGCCAGAAGAGAGGAGU	342	6139	CUAGCCAGAAGAGAGGAGU	342	6161	ACUCCUCUCUUCUGGCUAG	769
6157	UGGGACAGUCCUCUCCACC	343	6157	UGGGACAGUCCUCUCCACC	343	6179	GGUGGAGAGGACUGUCCCA	770
6175	CAAGAUCUAAAUCCAAACA	344	6175	CAAGAUCUAAAUCCAAACA	344	6197	UGUUUGGAUUUAGAUCUUG	771
6193	AAAAGCAGGCUAGAGCCAG	345	6193	AAAAGCAGGCUAGAGCCAG	345	6215	CUGGCUCUAGCCUGCUUUU	772
6211	GAAGAGAGGACAAAUCUUU	346	6211	GAAGAGAGGACAAAUCUUU	346	6233	AAAGAUUUGUCCUCUCUUC	773
6229	UGUUGUUCCUCUUCUUUAC	347	6229	UGUUGUUCCUCUUCUUUAC	347	6251	GUAAAGAAGAGGAACAACA	774
6247	CACAUACGCAAACCACCUG	348	6247	CACAUACGCAAACCACCUG	348	6269	CAGGUGGUUUGCGUAUGUG	775
6265	GUGACAGCUGGCAAUUUUA	349	6265	GUGACAGCUGGCAAUUUUA	349	6287	UAAAAUUGCCAGCUGUCAC	776
6283	AUAAAUCAGGUAACUGGAA	350	6283	AUAAAUCAGGUAACUGGAA	350	6305	UUCCAGUUACCUGAUUUAU	777
6301	AGGAGGUUAAACUCAGAAA	351	6301	AGGAGGUUAAACUCAGAAA	351	6323	UUUCUGAGUUUAACCUCCU	778
6319	AAAAGAAGACCUCAGUCAA	352	6319	AAAAGAAGACCUCAGUCAA	352	6341	UUGACUGAGGUCUUCUUUU	779
6337	AUUCUCUACUUUUUUUUUU	353	6337	AUUCUCUACUUUUUUUUUU	353	6359	AAAAAAAAAAGUAGAGAAU	780
6355	UUUUUUUCCAAAUCAGAUA	354	6355	UUUUUUUCCAAAUCAGAUA	354	6377	UAUCUGAUUUGGAAAAAAA	781

6373	AAUAGCCCAGCAAAUAGUG	355	6373	AAUAGCCCAGCAAAUAGUG	355	6395	CACUAUUUGCUGGGCUAUU	782
6391	GAUAACAAAUAAAACCUUA	356	6391	GAUAACAAAUAAAACCUUA	356	6413	UAAGGUUUUAUUUGUUAUC	783
6409	AGCUGUUCAUGUCUUGAUU	357	6409	AGCUGUUCAUGUCUUGAUU	357	6431	AAUCAAGACAUGAACAGCU	784
6427	UUCAAUAAUUAAUUCUUAA	358	6427	UUCAAUAAUUAAUUCUUAA	358	6449	UUAAGAAUUAAUUAUUGAA	785
6445	AUCAUUAAGAGACCAUAAU	359	6445	AUCAUUAAGAGACCAUAAU	359	6467	AUUAUGGUCUCUUAAUGAU	786
6463	UAAAUACUCCUUUUCAAGA	360	6463	UAAAUACUCCUUUUCAAGA	360	6485	UCUUGAAAAGGAGUAUUUA	787
6481	AGAAAAGCAAAACCAUUAG	361	6481	AGAAAAGCAAAACCAUUAG	361	6503	CUAAUGGUUUUGCUUUUCU	788
6499	GAAUUGUUACUCAGCUCCU	362	6499	GAAUUGUUACUCAGCUCCU	362	6521	AGGAGCUGAGUAACAAUUC	789
6517	UUCAAACUCAGGUUUGUAG	363	6517	UUCAAACUCAGGUUUGUAG	363	6539	CUACAAACCUGAGUUUGAA	790
6535	GCAUACAUGAGUCCAUCCA	364	6535	GCAUACAUGAGUCCAUCCA	364	6557	UGGAUGGACUCAUGUAUGC	791
6553	AUCAGUCAAAGAAUGGUUC	365	6553	AUCAGUCAAAGAAUGGUUC	365	6575	GAACCAUUCUUUGACUGAU	792
6571	CCAUCUGGAGUCUUAAUGU	366	6571	CCAUCUGGAGUCUUAAUGU	366	6593	ACAUUAAGACUCCAGAUGG	793
6589	UAGAAAGAAAAAUGGAGAC	367	6589	UAGAAAGAAAAAUGGAGAC	367	6611	GUCUCCAUUUUUCUUUCUA	794
6607	CUUGUAAUAAUGAGCUAGU	368	6607	CUUGUAAUAAUGAGCUAGU	368	6629	ACUAGCUCAUUAUUACAAG	795
6625	UUACAAAGUGCUUGUUCAU	369	6625	UUACAAAGUGCUUGUUCAU	369	6647	AUGAACAAGCACUUUGUAA	796
6643	UUAAAAUAGCACUGAAAAU	370	6643	UUAAAAUAGCACUGAAAAU	370	6665	AUUUUCAGUGCUAUUUUAA	797
6661	UUGAAACAUGAAUUAACUG	371	6661	UUGAAACAUGAAUUAACUG	371	6683	CAGUUAAUUCAUGUUUCAA	798
6679	GAUAAUAUUCCAAUCAUUU	372	6679	GAUAAUAUUCCAAUCAUUU	372	6701	AAAUGAUUGGAAUAUUAUC	799
6697	UGCCAUUUAUGACAAAAAU	373	6697	UGCCAUUUAUGACAAAAAU	373	6719	AUUUUUGUCAUAAAUGGCA	800
6715	UGGUUGGCACUAACAAAGA	374	6715	UGGUUGGCACUAACAAAGA	374	6737	UCUUUGUUAGUGCCAACCA	801
6733	AACGAGCACUUCCUUUCAG	375	6733	AACGAGCACUUCCUUUCAG	375	6755	CUGAAAGGAAGUGCUCGUU	802
6751	GAGUUUCUGAGAUAAUGUA	376	6751	GAGUUUCUGAGAUAAUGUA	376	6773	UACAUUAUCUCAGAAACUC	803
6769	ACGUGGAACAGUCUGGGUG	377	6769	ACGUGGAACAGUCUGGGUG	377	6791	CACCCAGACUGUUCCACGU	804
6787	GGAAUGGGGCUGAAACCAU	378	6787	GGAAUGGGGCUGAAACCAU	378	6809	AUGGUUUCAGCCCCAUUCC	805
6805	UGUGCAAGUCUGUGUCUUG	379	6805	UGUGCAAGUCUGUGUCUUG	379	6827	CAAGACACAGACUUGCACA	806
6823	GUCAGUCCAAGAAGUGACA	380	6823	GUCAGUCCAAGAAGUGACA	380	6845	UGUCACUUCUUGGACUGAC	807
6841	ACCGAGAUGUUAAUUUUAG	381	6841	ACCGAGAUGUUAAUUUUAG	381	6863	CUAAAAUUAACAUCUCGGU	808
6859	GGGACCCGUGCCUUGUUUC	382	6859	GGGACCCGUGCCUUGUUUC	382	6881	GAAACAAGGCACGGGUCCC	809
6877	CCUAGCCCACAAGAAUGCA	383	6877	CCUAGCCCACAAGAAUGCA	383	6899	UGCAUUCUUGUGGGCUAGG	810
6895	AAACAUCAAACAGAUACUC	384	6895	AAACAUCAAACAGAUACUC	384	6917	GAGUAUCUGUUUGAUGUUU	811
6913	CGCUAGCCUCAUUUAAAUU	385	6913	CGCUAGCCUCAUUUAAAUU	385	6935	AAUUUAAAUGAGGCUAGCG	812
6931	UGAUUAAAGGAGGAGUGCA	386	6931	UGAUUAAAGGAGGAGUGCA	386	6953	UGCACUCCUCCUUUAAUCA	813
6949	AUCUUUGGCCGACAGUGGU	387	6949	AUCUUUGGCCGACAGUGGU	387	6971	ACCACUGUCGGCCAAAGAU	814
6967	UGUAACUGUGUGUGUGUGU	388	6967	UGUAACUGUGUGUGUGUGU	388	6989	ACACACACACACAGUUACA	815
6985	UGUGUGUGUGUGUGUGUGU	389	6985	UGUGUGUGUGUGUGUGUGU	389	7007	ACACACACACACACACACA	816
7003	UGUGUGUGUGUGGGUGUGG	390	7003	UGUGUGUGUGUGGGUGUGG	390	7025	CCACACCCACACACACACA	817

7021	GGUGUAUGUGUGUUUUGUG	391	7021	GGUGUAUGUGUGUUUUGUG	391	7043	CACAAAACACACAUACACC	818
7039	GCAUAACUAUUUAAGGAAA	392	7039	GCAUAACUAUUUAAGGAAA	392	7061	UUUCCUUAAAUAGUUAUGC	819
7057	ACUGGAAUUUUAAAGUUAC	393	7057	ACUGGAAUUUUAAAGUUAC	393	7079	GUAACUUUAAAAUUCCAGU	820
7075	CUUUUAUACAAACCAAGAA	394	7075	CUUUUAUACAAACCAAGAA	394	7097	UUCUUGGUUUGUAUAAAAG	821
7093	AUAUAUGCUACAGAUAUAA	395	7093	AUAUAUGCUACAGAUAUAA	395	7115	UUAUAUCUGUAGCAUAUAU	822
7111	AGACAGACAUGGUUUGGUC	396	7111	AGACAGACAUGGUUUGGUC	396	7133	GACCAAACCAUGUCUGUCU	823
7129	CCUAUAUUUCUAGUCAUGA	397	7129	CCUAUAUUUCUAGUCAUGA	397	7151	UCAUGACUAGAAAUAUAGG	824
7147	AUGAAUGUAUUUUGUAUAC	398	7147	AUGAAUGUAUUUUGUAUAC	398	7169	GUAUACAAAAUACAUUCAU	825
7165	CCAUCUUCAUAUAAUAUAC	399	7165	CCAUCUUCAUAUAAUAUAC	399	7187	GUAUAUUAUAUGAAGAUGG	826
7183	CUUAAAAAUAUUUCUUAAU	400	7183	CUUAAAAAUAUUUCUUAAU	400	7205	AUUAAGAAAUAUUUUUAAG	827
7201	UUGGGAUUUGUAAUCGUAC	401	7201	UUGGGAUUUGUAAUCGUAC	401	7223	GUACGAUUACAAAUCCCAA	828
7219	CCAACUUAAUUGAUAAACU	402	7219	CCAACUUAAUUGAUAAACU	402	7241	AGUUUAUCAAUUAAGUUGG	829
7237	UUGGCAACUGCUUUUAUGU	403	7237	UUGGCAACUGCUUUUAUGU	403	7259	ACAUAAAAGCAGUUGCCAA	830
7255	UUCUGUCUCCUUCCAUAAA	404	7255	UUCUGUCUCCUUCCAUAAA	404	7277	UUUAUGGAAGGAGACAGAA	831
7273	AUUUUUCAAAAUACUAAUU	405	7273	AUUUUUCAAAAUACUAAUU	405	7295	AAUUAGUAUUUUGAAAAAU	832
7291	UCAACAAAGAAAAAGCUCU	406	7291	UCAACAAAGAAAAAGCUCU	406	7313	AGAGCUUUUUCUUUGUUGA	833
7309	UUUUUUUUCCUAAAAUAAA	407	7309	UUUUUUUUCCUAAAAUAAA	407	7331	UUUAUUUUAGGAAAAAAAA	834
7327	ACUCAAAUUUAUCCUUGUU	408	7327	ACUCAAAUUUAUCCUUGUU	408	7349	AACAAGGAUAAAUUUGAGU	835
7345	UUAGAGCAGAGAAAAAUUA	409	7345	UUAGAGCAGAGAAAAAUUA	409	7367	UAAUUUUUCUCUGCUCUAA	836
7363	AAGAAAAACUUUGAAAUGG	410	7363	AAGAAAAACUUUGAAAUGG	410	7385	CCAUUUCAAAGUUUUUCUU	837
7381	GUCUCAAAAAAUUGCUAAA	411	7381	GUCUCAAAAAAUUGCUAAA	411	7403	UUUAGCAAUUUUUUGAGAC	838
7399	AUAUUUUCAAUGGAAAACU	412	7399	AUAUUUUCAAUGGAAAACU	412	7421	AGUUUUCCAUUGAAAAUAU	839
7417	UAAAUGUUAGUUUAGCUGA	413	7417	UAAAUGUUAGUUUAGCUGA	413	7439	UCAGCUAAACUAACAUUUA	840
7435	AUUGUAUGGGGUUUUCGAA	414	7435	AUUGUAUGGGGUUUUCGAA	414	7457	UUCGAAAACCCCAUACAAU	841
7453	ACCUUUCACUUUUUGUUUG	415	7453	ACCUUUCACUUUUUGUUUG	415	7475	CAAACAAAAAGUGAAAGGU	842
7471	GUUUUACCUAUUUCACAAC	416	7471	GUUUUACCUAUUUCACAAC	416	7493	GUUGUGAAAUAGGUAAAAC	843
7489	CUGUGUAAAUUGCCAAUAA	417	7489	CUGUGUAAAUUGCCAAUAA	417	7511	UUAUUGGCAAUUUACACAG	844
7507	AUUCCUGUCCAUGAAAAUG	418	7507	AUUCCUGUCCAUGAAAAUG	418	7529	CAUUUUCAUGGACAGGAAU	845
7525	GCAAAUUAUCCAGUGUAGA	419	7525	GCAAAUUAUCCAGUGUAGA	419	7547	UCUACACUGGAUAAUUUGC	846
7543	AUAUAUUUGACCAUCACCC	420	7543	AUAUAUUUGACCAUCACCC	420	7565	GGGUGAUGGUCAAAUAUAU	847
7561	CUAUGGAUAUUGGCUAGUU	421	7561	CUAUGGAUAUUGGCUAGUU	421	7583	AACUAGCCAAUAUCCAUAG	848
7579	UUUGCCUUUAUUAAGCAAA	422	7579	UUUGCCUUUAUUAAGCAAA	422	7601	UUUGCUUAAUAAAGGCAAA	849
7597	AUUCAUUUCAGCCUGAAUG	423	7597	AUUCAUUUCAGCCUGAAUG	423	7619	CAUUCAGGCUGAAAUGAAU	850
7615	GUCUGCCUAUAUAUUCUCU	424	7615	GUCUGCCUAUAUAUUCUCU	424	7637	AGAGAAUAUAUAGGCAGAC	851
7633	UGCUCUUUGUAUUCUCCUU	425	7633	UGCUCUUUGUAUUCUCCUU	425	7655	AAGGAGAAUACAAAGAGCA	852
7651	UUGAACCCGUUAAAACAUC	426	7651	UUGAACCCGUUAAAACAUC	426	7673	GAUGUUUUAACGGGUUCAA	853

7662	AAAACAUCCUGUGGCACUC	427	7662	AAAACAUCCUGUGGCACUC	427	7684	GAGUGCCACAGGAUGUUUU	854

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Pos	Target Sequence	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
1	ACUGAGUCCCGGGACCCCG	855	1	ACUGAGUCCCGGGACCCCG	855	23	CGGGGUCCCGGGACUCAGU	1179
19	GGGAGAGCGGUCAGUGUGU	856	19	GGGAGAGCGGUCAGUGUGU	856	41	ACACACUGACCGCUCUCCC	1180
37	UGGUCGCUGCGUUUCCUCU	857	37	UGGUCGCUGCGUUUCCUCU	857	59	AGAGGAAACGCAGCGACCA	1181
55	UGCCUGCGCCGGGCAUCAC	858	55	UGCCUGCGCCGGGCAUCAC	858	77	GUGAUGCCCGGCGCAGGCA	1182
73	CUUGCGCGCCGCAGAAAGU	859	73	CUUGCGCGCCGCAGAAAGU	859	95	ACUUUCUGCGGCGCGCAAG	1183
91	UCCGUCUGGCAGCCUGGAU	860	91	UCCGUCUGGCAGCCUGGAU	860	113	AUCCAGGCUGCCAGACGGA	1184
109	UAUCCUCUCCUACCGGCAC	861	109	UAUCCUCUCCUACCGGCAC	861	131	GUGCCGGUAGGAGAGGAUA	1185
127	CCCGCAGACGCCCCUGCAG	862	127	CCCGCAGACGCCCCUGCAG	862	149	CUGCAGGGGCGUCUGCGGG	1186
145	GCCGCCGGUCGGCGCCCGG	863	145	GCCGCCGGUCGGCGCCCGG	863	167	CCGGGCGCCGACCGGCGGC	1187
163	GGCUCCCUAGCCCUGUGCG	864	163	GGCUCCCUAGCCCUGUGCG	864	185	CGCACAGGGCUAGGGAGCC	1188
181	GCUCAACUGUCCUGCGCUG	865	181	GCUCAACUGUCCUGCGCUG	865	203	CAGCGCAGGACAGUUGAGC	1189
199	GCGGGGUGCCGCGAGUUCC	866	199	GCGGGGUGCCGCGAGUUCC	866	221	GGAACUCGCGGCACCCCGC	1190
217	CACCUCCGCGCCUCCUUCU	867	217	CACCUCCGCGCCUCCUUCU	867	239	AGAAGGAGGCGCGGAGGUG	1191
235	UCUAGACAGGCGCUGGGAG	868	235	UCUAGACAGGCGCUGGGAG	868	257	CUCCCAGCGCCUGUCUAGA	1192
253	GAAAGAACCGGCUCCCGAG	869	253	GAAAGAACCGGCUCCCGAG	869	275	CUCGGGAGCCGGUUCUUUC	1193
271	GUUCUGGGCAUUUCGCCCG	870	271	GUUCUGGGCAUUUCGCCCG	870	293	CGGGCGAAAUGCCCAGAAC	1194
289	GGCUCGAGGUGCAGGAUGC	871	289	GGCUCGAGGUGCAGGAUGC	871	311	GCAUCCUGCACCUCGAGCC	1195
307	CAGAGCAAGGUGCUGCUGG	872	307	CAGAGCAAGGUGCUGCUGG	872	329	CCAGCAGCACCUUGCUCUG	1196
325	GCCGUCGCCCUGUGGCUCU	873	325	GCCGUCGCCCUGUGGCUCU	873	347	AGAGCCACAGGGCGACGGC	1197
343	UGCGUGGAGACCCGGGCCG	874	343	UGCGUGGAGACCCGGGCCG	874	365	CGGCCCGGGUCUCCACGCA	1198
361	GCCUCUGUGGGUUUGCCUA	875	361	GCCUCUGUGGGUUUGCCUA	875	383	UAGGCAAACCCACAGAGGC	1199
379	AGUGUUUCUCUUGAUCUGC	876	379	AGUGUUUCUCUUGAUCUGC	876	401	GCAGAUCAAGAGAAACACU	1200
397	CCCAGGCUCAGCAUACAAA	877	397	CCCAGGCUCAGCAUACAAA	877	419	UUUGUAUGCUGAGCCUGGG	1201
415	AAAGACAUACUUACAAUUA	878	415	AAAGACAUACUUACAAUUA	878	437	UAAUUGUAAGUAUGUCUUU	1202
433	AAGGCUAAUACAACUCUUC	879	433	AAGGCUAAUACAACUCUUC	879	455	GAAGAGUUGUAUUAGCCUU	1203
451	CAAAUUACUUGCAGGGGAC	880	451	CAAAUUACUUGCAGGGGAC	880	473	GUCCCCUGCAAGUAAUUUG	1204
469	CAGAGGGACUUGGACUGGC	881	469	CAGAGGGACUUGGACUGGC	881	491	GCCAGUCCAAGUCCCUCUG	1205
487	CUUUGGCCCAAUAAUCAGA	882	487	CUUUGGCCCAAUAAUCAGA	882	509	UCUGAUUAUUGGGCCAAAG	1206
505	AGUGGCAGUGAGCAAAGGG	883	505	AGUGGCAGUGAGCAAAGGG	883	527	CCCUUUGCUCACUGCCACU	1207
523	GUGGAGGUGACUGAGUGCA	884	523	GUGGAGGUGACUGAGUGCA	884	545	UGCACUCAGUCACCUCCAC	1208

541	AGCGAUGGCCUCUUCUGUA	885	541	AGCGAUGGCCUCUUCUGUA	885	563	UACAGAAGAGGCCAUCGCU	1209
559	AAGACACUCACAAUUCCAA	886	559	AAGACACUCACAAUUCCAA	886	581	UUGGAAUUGUGAGUGUCUU	1210
577	AAAGUGAUCGGAAAUGACA	887	577	AAAGUGAUCGGAAAUGACA	887	599	UGUCAUUUCCGAUCACUUU	1211
595	ACUGGAGCCUACAAGUGCU	888	595	ACUGGAGCCUACAAGUGCU	888	617	AGCACUUGUAGGCUCCAGU	1212
613	UUCUACCGGGAAACUGACU	889	613	UUCUACCGGGAAACUGACU	889	635	AGUCAGUUUCCCGGUAGAA	1213
631	UUGGCCUCGGUCAUUUAUG	890	631	UUGGCCUCGGUCAUUUAUG	890	653	CAUAAAUGACCGAGGCCAA	1214
649	GUCUAUGUUCAAGAUUACA	891	649	GUCUAUGUUCAAGAUUACA	891	671	UGUAAUCUUGAACAUAGAC	1215
667	AGAUCUCCAUUUAUUGCUU	892	667	AGAUCUCCAUUUAUUGCUU	892	689	AAGCAAUAAAUGGAGAUCU	1216
685	UCUGUUAGUGACCAACAUG	893	685	UCUGUUAGUGACCAACAUG	893	707	CAUGUUGGUCACUAACAGA	1217
703	GGAGUCGUGUACAUUACUG	894	703	GGAGUCGUGUACAUUACUG	894	725	CAGUAAUGUACACGACUCC	1218
721	GAGAACAAAAACAAAACUG	895	721	GAGAACAAAAACAAAACUG	895	743	CAGUUUUGUUUUUGUUCUC	1219
739	GUGGUGAUUCCAUGUCUCG	896	739	GUGGUGAUUCCAUGUCUCG	896	761	CGAGACAUGGAAUCACCAC	1220
757	GGGUCCAUUUCAAAUCUCA	897	757	GGGUCCAUUUCAAAUCUCA	897	779	UGAGAUUUGAAAUGGACCC	1221
775	AACGUGUCACUUUGUGCAA	898	775	AACGUGUCACUUUGUGCAA	898	797	UUGCACAAAGUGACACGUU	1222
793	AGAUACCCAGAAAAGAGAU	899	793	AGAUACCCAGAAAAGAGAU	899	815	AUCUCUUUUCUGGGUAUCU	1223
811	UUUGUUCCUGAUGGUAACA	900	811	UUUGUUCCUGAUGGUAACA	900	833	UGUUACCAUCAGGAACAAA	1224
829	AGAAUUUCCUGGGACAGCA	901	829	AGAAUUUCCUGGGACAGCA	901	851	UGCUGUCCCAGGAAAUUCU	1225
847	AAGAAGGGCUUUACUAUUC	902	847	AAGAAGGGCUUUACUAUUC	902	869	GAAUAGUAAAGCCCUUCUU	1226
865	CCCAGCUACAUGAUCAGCU	903	865	CCCAGCUACAUGAUCAGCU	903	887	AGCUGAUCAUGUAGCUGGG	1227
883	UAUGCUGGCAUGGUCUUCU	904	883	UAUGCUGGCAUGGUCUUCU	904	905	AGAAGACCAUGCCAGCAUA	1228
901	UGUGAAGCAAAAAUUAAUG	905	901	UGUGAAGCAAAAAUUAAUG	905	923	CAUUAAUUUUUGCUUCACA	1229
919	GAUGAAAGUUACCAGUCUA	906	919	GAUGAAAGUUACCAGUCUA	906	941	UAGACUGGUAACUUUCAUC	1230
937	AUUAUGUACAUAGUUGUCG	907	937	AUUAUGUACAUAGUUGUCG	907	959	CGACAACUAUGUACAUAAU	1231
955	GUUGUAGGGUAUAGGAUUU	908	955	GUUGUAGGGUAUAGGAUUU	908	977	AAAUCCUAUACCCUACAAC	1232
973	UAUGAUGUGGUUCUGAGUC	909	973	UAUGAUGUGGUUCUGAGUC	909	995	GACUCAGAACCACAUCAUA	1233
991	CCGUCUCAUGGAAUUGAAC	910	991	CCGUCUCAUGGAAUUGAAC	910	1013	GUUCAAUUCCAUGAGACGG	1234
1009	CUAUCUGUUGGAGAAAAGC	911	1009	CUAUCUGUUGGAGAAAAGC	911	1031	GCUUUUCUCCAACAGAUAG	1235
1027	CUUGUCUUAAAUUGUACAG	912	1027	CUUGUCUUAAAUUGUACAG	912	1049	CUGUACAAUUUAAGACAAG	1236
1045	GCAAGAACUGAACUAAAUG	913	1045	GCAAGAACUGAACUAAAUG	913	1067	CAUUUAGUUCAGUUCUUGC	1237
1063	GUGGGGAUUGACUUCAACU	914	1063	GUGGGGAUUGACUUCAACU	914	1085	AGUUGAAGUCAAUCCCCAC	1238
1081	UGGGAAUACCCUUCUUCGA	915	1081	UGGGAAUACCCUUCUUCGA	915	1103	UCGAAGAAGGGUAUUCCCA	1239
1099	AAGCAUCAGCAUAAGAAAC	916	1099	AAGCAUCAGCAUAAGAAAC	916	1121	GUUUCUUAUGCUGAUGCUU	1240
1117	CUUGUAAACCGAGACCUAA	917	1117	CUUGUAAACCGAGACCUAA	917	1139	UUAGGUCUCGGUUUACAAG	1241
1135	AAAACCCAGUCUGGGAGUG	918	1135	AAAACCCAGUCUGGGAGUG	918	1157	CACUCCCAGACUGGGUUUU	1242
1153	GAGAUGAAGAAAUUUUUGA	919	1153	GAGAUGAAGAAAUUUUUGA	919	1175	UCAAAAAUUUCUUCAUCUC	1243
1171	AGCACCUUAACUAUAGAUG	920	1171	AGCACCUUAACUAUAGAUG	920	1193	CAUCUAUAGUUAAGGUGCU	1244

1189	GGUGUAACCCGGAGUGACC	921	1189	GGUGUAACCCGGAGUGACC	921	1211	GGUCACUCCGGGUUACACC	1245
1207	CAAGGAUUGUACACCUGUG	922	1207	CAAGGAUUGUACACCUGUG	922	1229	CACAGGUGUACAAUCCUUG	1246
1225	GCAGCAUCCAGUGGGCUGA	923	1225	GCAGCAUCCAGUGGGCUGA	923	1247	UCAGCCCACUGGAUGCUGC	1247
1243	AUGACCAAGAAGAACAGCA	924	1243	AUGACCAAGAAGAACAGCA	924	1265	UGCUGUUCUUCUUGGUCAU	1248
1261	ACAUUUGUCAGGGUCCAUG	925	1261	ACAUUUGUCAGGGUCCAUG	925	1283	CAUGGACCCUGACAAAUGU	1249
1279	GAAAAACCUUUUGUUGCUU	926	1279	GAAAAACCUUUUGUUGCUU	926	1301	AAGCAACAAAAGGUUUUUC	1250
1297	UUUGGAAGUGGCAUGGAAU	927	1297	UUUGGAAGUGGCAUGGAAU	927	1319	AUUCCAUGCCACUUCCAAA	1251
1315	UCUCUGGUGGAAGCCACGG	928	1315	UCUCUGGUGGAAGCCACGG	928	1337	CCGUGGCUUCCACCAGAGA	1252
1333	GUGGGGGAGCGUGUCAGAA	929	1333	GUGGGGGAGCGUGUCAGAA	929	1355	UUCUGACACGCUCCCCCAC	1253
1351	AUCCCUGCGAAGUACCUUG	930	1351	AUCCCUGCGAAGUACCUUG	930	1373	CAAGGUACUUCGCAGGGAU	1254
1369	GGUUACCCACCCCCAGAAA	931	1369	GGUUACCCACCCCCAGAAA	931	1391	UUUCUGGGGGUGGGUAACC	1255
1387	AUAAAAUGGUAUAAAAAUG	932	1387	AUAAAAUGGUAUAAAAAUG	932	1409	CAUUUUUAUACCAUUUUAU	1256
1405	GGAAUACCCCUUGAGUCCA	933	1405	GGAAUACCCCUUGAGUCCA	933	1427	UGGACUCAAGGGGUAUUCC	1257
1423	AAUCACACAAUUAAAGCGG	934	1423	AAUCACACAAUUAAAGCGG	934	1445	CCGCUUUAAUUGUGUGAUU	1258
1441	GGGCAUGUACUGACGAUUA	935	1441	GGGCAUGUACUGACGAUUA	935	1463	UAAUCGUCAGUACAUGCCC	1259
1459	AUGGAAGUGAGUGAAAGAG	936	1459	AUGGAAGUGAGUGAAAGAG	936	1481	CUCUUUCACUCACUUCCAU	1260
1477	GACACAGGAAAUUACACUG	937	1477	GACACAGGAAAUUACACUG	937	1499	CAGUGUAAUUUCCUGUGUC	1261
1495	GUCAUCCUUACCAAUCCCA	938	1495	GUCAUCCUUACCAAUCCCA	938	1517	UGGGAUUGGUAAGGAUGAC	1262
1513	AUUUCAAAGGAGAAGCAGA	939	1513	AUUUCAAAGGAGAAGCAGA	939	1535	UCUGCUUCUCCUUUGAAAU	1263
1531	AGCCAUGUGGUCUCUCUGG	940	1531	AGCCAUGUGGUCUCUCUGG	940	1553	CCAGAGAGACCACAUGGCU	1264
1549	GUUGUGUAUGUCCCACCCC	941	1549	GUUGUGUAUGUCCCACCCC	941	1571	GGGGUGGGACAUACACAAC	1265
1567	CAGAUUGGUGAGAAAUCUC	942	1567	CAGAUUGGUGAGAAAUCUC	942	1589	GAGAUUUCUCACCAAUCUG	1266
1585	CUAAUCUCUCCUGUGGAUU	943	1585	CUAAUCUCUCCUGUGGAUU	943	1607	AAUCCACAGGAGAGAUUAG	1267
1603	UCCUACCAGUACGGCACCA	944	1603	UCCUACCAGUACGGCACCA	944	1625	UGGUGCCGUACUGGUAGGA	1268
1621	ACUCAAACGCUGACAUGUA	945	1621	ACUCAAACGCUGACAUGUA	945	1643	UACAUGUCAGCGUUUGAGU	1269
1639	ACGGUCUAUGCCAUUCCUC	946	1639	ACGGUCUAUGCCAUUCCUC	946	1661	GAGGAAUGGCAUAGACCGU	1270
1657	CCCCCGCAUCACAUCCACU	947	1657	CCCCCGCAUCACAUCCACU	947	1679	AGUGGAUGUGAUGCGGGGG	1271
1675	UGGUAUUGGCAGUUGGAGG	948	1675	UGGUAUUGGCAGUUGGAGG	948	1697	CCUCCAACUGCCAAUACCA	1272
1693	GAAGAGUGCGCCAACGAGC	949	1693	GAAGAGUGCGCCAACGAGC	949	1715	GCUCGUUGGCGCACUCUUC	1273
1711	CCCAGCCAAGCUGUCUCAG	950	1711	CCCAGCCAAGCUGUCUCAG	950	1733	CUGAGACAGCUUGGCUGGG	1274
1729	GUGACAAACCCAUACCCUU	951	1729	GUGACAAACCCAUACCCUU	951	1751	AAGGGUAUGGGUUUGUCAC	1275
1747	UGUGAAGAAUGGAGAAGUG	952	1747	UGUGAAGAAUGGAGAAGUG	952	1769	CACUUCUCCAUUCUUCACA	1276
1765	GUGGAGGACUUCCAGGGAG	953	1765	GUGGAGGACUUCCAGGGAG	953	1787	CUCCCUGGAAGUCCUCCAC	1277
1783	GGAAAUAAAAUUGAAGUUA	954	1783	GGAAAUAAAAUUGAAGUUA	954	1805	UAACUUCAAUUUUAUUUCC	1278
1801	AAUAAAAAUCAAUUUGCUC	955	1801	AAUAAAAAUCAAUUUGCUC	955	1823	GAGCAAAUUGAUUUUUAUU	1279
1819	CUAAUUGAAGGAAAAAACA	956	1819	CUAAUUGAAGGAAAAAACA	956	1841	UGUUUUUUCCUUCAAUUAG	1280

1837	AAAACUGUAAGUACCCUUG	957	1837	AAAACUGUAAGUACCCUUG	957	1859	CAAGGGUACUUACAGUUUU	1281
1855	GUUAUCCAAGCGGCAAAUG	958	1855	GUUAUCCAAGCGGCAAAUG	958	1877	CAUUUGCCGCUUGGAUAAC	1282
1873	GUGUCAGCUUUGUACAAAU	959	1873	GUGUCAGCUUUGUACAAAU	959	1895	AUUUGUACAAAGCUGACAC	1283
1891	UGUGAAGCGGUCAACAAAG	960	1891	UGUGAAGCGGUCAACAAAG	960	1913	CUUUGUUGACCGCUUCACA	1284
1909	GUCGGGAGAGGAGAGAGGG	961	1909	GUCGGGAGAGGAGAGAGGG	961	1931	CCCUCUCUCCUCUCCCGAC	1285
1927	GUGAUCUCCUUCCACGUGA	962	1927	GUGAUCUCCUUCCACGUGA	962	1949	UCACGUGGAAGGAGAUCAC	1286
1945	ACCAGGGGUCCUGAAAUUA	963	1945	ACCAGGGGUCCUGAAAUUA	963	1967	UAAUUUCAGGACCCCUGGU	1287
1963	ACUUUGCAACCUGACAUGC	964	1963	ACUUUGCAACCUGACAUGC	964	1985	GCAUGUCAGGUUGCAAAGU	1288
1981	CAGCCCACUGAGCAGGAGA	965	1981	CAGCCCACUGAGCAGGAGA	965	2003	UCUCCUGCUCAGUGGGCUG	1289
1999	AGCGUGUCUUUGUGGUGCA	966	1999	AGCGUGUCUUUGUGGUGCA	966	2021	UGCACCACAAAGACACGCU	1290
2017	ACUGCAGACAGAUCUACGU	967	2017	ACUGCAGACAGAUCUACGU	967	2039	ACGUAGAUCUGUCUGCAGU	1291
2035	UUUGAGAACCUCACAUGGU	968	2035	UUUGAGAACCUCACAUGGU	968	2057	ACCAUGUGAGGUUCUCAAA	1292
2053	UACAAGCUUGGCCCACAGC	969	2053	UACAAGCUUGGCCCACAGC	969	2075	GCUGUGGGCCAAGCUUGUA	1293
2071	CCUCUGCCAAUCCAUGUGG	970	2071	CCUCUGCCAAUCCAUGUGG	970	2093	CCACAUGGAUUGGCAGAGG	1294
2089	GGAGAGUUGCCCACACCUG	971	2089	GGAGAGUUGCCCACACCUG	971	2111	CAGGUGUGGGCAACUCUCC	1295
2107	GUUUGCAAGAACUUGGAUA	972	2107	GUUUGCAAGAACUUGGAUA	972	2129	UAUCCAAGUUCUUGCAAAC	1296
2125	ACUCUUUGGAAAUUGAAUG	973	2125	ACUCUUUGGAAAUUGAAUG	973	2147	CAUUCAAUUUCCAAAGAGU	1297
2143	GCCACCAUGUUCUCUAAUA	974	2143	GCCACCAUGUUCUCUAAUA	974	2165	UAUUAGAGAACAUGGUGGC	1298
2161	AGCACAAAUGACAUUUUGA	975	2161	AGCACAAAUGACAUUUUGA	975	2183	UCAAAAUGUCAUUUGUGCU	1299
2179	AUCAUGGAGCUUAAGAAUG	976	2179	AUCAUGGAGCUUAAGAAUG	976	2201	CAUUCUUAAGCUCCAUGAU	1300
2197	GCAUCCUUGCAGGACCAAG	977	2197	GCAUCCUUGCAGGACCAAG	977	2219	CUUGGUCCUGCAAGGAUGC	1301
2215	GGAGACUAUGUCUGCCUUG	978	2215	GGAGACUAUGUCUGCCUUG	978	2237	CAAGGCAGACAUAGUCUCC	1302
2233	GCUCAAGACAGGAAGACCA	979	2233	GCUCAAGACAGGAAGACCA	979	2255	UGGUCUUCCUGUCUUGAGC	1303
2251	AAGAAAAGACAUUGCGUGG	980	2251	AAGAAAAGACAUUGCGUGG	980	2273	CCACGCAAUGUCUUUUCUU	1304
2269	GUCAGGCAGCUCACAGUCC	981	2269	GUCAGGCAGCUCACAGUCC	981	2291	GGACUGUGAGCUGCCUGAC	1305
2287	CUAGAGCGUGUGGCACCCA	982	2287	CUAGAGCGUGUGGCACCCA	982	2309	UGGGUGCCACACGCUCUAG	1306
2305	ACGAUCACAGGAAACCUGG	983	2305	ACGAUCACAGGAAACCUGG	983	2327	CCAGGUUUCCUGUGAUCGU	1307
2323	GAGAAUCAGACGACAAGUA	984	2323	GAGAAUCAGACGACAAGUA	984	2345	UACUUGUCGUCUGAUUCUC	1308
2341	AUUGGGGAAAGCAUCGAAG	985	2341	AUUGGGGAAAGCAUCGAAG	985	2363	CUUCGAUGCUUUCCCCAAU	1309
2359	GUCUCAUGCACGGCAUCUG	986	2359	GUCUCAUGCACGGCAUCUG	986	2381	CAGAUGCCGUGCAUGAGAC	1310
2377	GGGAAUCCCCCUCCACAGA	987	2377	GGGAAUCCCCCUCCACAGA	987	2399	UCUGUGGAGGGGGAUUCCC	1311
2395	AUCAUGUGGUUUAAAGAUA	988	2395	AUCAUGUGGUUUAAAGAUA	988	2417	UAUCUUUAAACCACAUGAU	1312
2413	AAUGAGACCCUUGUAGAAG	989	2413	AAUGAGACCCUUGUAGAAG	989	2435	CUUCUACAAGGGUCUCAUU	1313
2431	GACUCAGGCAUUGUAUUGA	990	2431	GACUCAGGCAUUGUAUUGA	990	2453	UCAAUACAAUGCCUGAGUC	1314
2449	AAGGAUGGGAACCGGAACC	991	2449	AAGGAUGGGAACCGGAACC	991	2471	GGUUCCGGUUCCCAUCCUU	1315
2467	CUCACUAUCCGCAGAGUGA	992	2467	CUCACUAUCCGCAGAGUGA	992	2489	UCACUCUGCGGAUAGUGAG	1316

2485	AGGAAGGAGGACGAAGGCC	993	2485	AGGAAGGAGGACGAAGGCC	993	2507	GGCCUUCGUCCUCCUUCCU	1317
2503	CUCUACACCUGCCAGGCAU	994	2503	CUCUACACCUGCCAGGCAU	994	2525	AUGCCUGGCAGGUGUAGAG	1318
2521	UGCAGUGUUCUUGGCUGUG	995	2521	UGCAGUGUUCUUGGCUGUG	995	2543	CACAGCCAAGAACACUGCA	1319
2539	GCAAAAGUGGAGGCAUUUU	996	2539	GCAAAAGUGGAGGCAUUUU	996	2561	AAAAUGCCUCCACUUUUGC	1320
2557	UUCAUAAUAGAAGGUGCCC	997	2557	UUCAUAAUAGAAGGUGCCC	997	2579	GGGCACCUUCUAUUAUGAA	1321
2575	CAGGAAAAGACGAACUUGG	998	2575	CAGGAAAAGACGAACUUGG	998	2597	CCAAGUUCGUCUUUUCCUG	1322
2593	GAAAUCAUUAUUCUAGUAG	999	2593	GAAAUCAUUAUUCUAGUAG	999	2615	CUACUAGAAUAAUGAUUUC	1323
2611	GGCACGGCGGUGAUUGCCA	1000	2611	GGCACGGCGGUGAUUGCCA	1000	2633	UGGCAAUCACCGCCGUGCC	1324
2629	AUGUUCUUCUGGCUACUUC	1001	2629	AUGUUCUUCUGGCUACUUC	1001	2651	GAAGUAGCCAGAAGAACAU	1325
2647	CUUGUCAUCAUCCUACGGA	1002	2647	CUUGUCAUCAUCCUACGGA	1002	2669	UCCGUAGGAUGAUGACAAG	1326
2665	ACCGUUAAGCGGGCCAAUG	1003	2665	ACCGUUAAGCGGGCCAAUG	1003	2687	CAUUGGCCCGCUUAACGGU	1327
2683	GGAGGGGAACUGAAGACAG	1004	2683	GGAGGGGAACUGAAGACAG	1004	2705	CUGUCUUCAGUUCCCCUCC	1328
2701	GGCUACUUGUCCAUCGUCA	1005	2701	GGCUACUUGUCCAUCGUCA	1005	2723	UGACGAUGGACAAGUAGCC	1329
2719	AUGGAUCCAGAUGAACUCC	1006	2719	AUGGAUCCAGAUGAACUCC	1006	2741	GGAGUUCAUCUGGAUCCAU	1330
2737	CCAUUGGAUGAACAUUGUG	1007	2737	CCAUUGGAUGAACAUUGUG	1007	2759	CACAAUGUUCAUCCAAUGG	1331
2755	GAACGACUGCCUUAUGAUG	1008	2755	GAACGACUGCCUUAUGAUG	1008	2777	CAUCAUAAGGCAGUCGUUC	1332
2773	GCCAGCAAAUGGGAAUUCC	1009	2773	GCCAGCAAAUGGGAAUUCC	1009	2795	GGAAUUCCCAUUUGCUGGC	1333
2791	CCCAGAGACCGGCUGAAGC	1010	2791	CCCAGAGACCGGCUGAAGC	1010	2813	GCUUCAGCCGGUCUCUGGG	1334
2809	CUAGGUAAGCCUCUUGGCC	1011	2809	CUAGGUAAGCCUCUUGGCC	1011	2831	GGCCAAGAGGCUUACCUAG	1335
2827	CGUGGUGCCUUUGGCCAAG	1012	2827	CGUGGUGCCUUUGGCCAAG	1012	2849	CUUGGCCAAAGGCACCACG	1336
2845	GUGAUUGAAGCAGAUGCCU	1013	2845	GUGAUUGAAGCAGAUGCCU	1013	2867	AGGCAUCUGCUUCAAUCAC	1337
2863	UUUGGAAUUGACAAGACAG	1014	2863	UUUGGAAUUGACAAGACAG	1014	2885	CUGUCUUGUCAAUUCCAAA	1338
2881	GCAACUUGCAGGACAGUAG	1015	2881	GCAACUUGCAGGACAGUAG	1015	2903	CUACUGUCCUGCAAGUUGC	1339
2899	GCAGUCAAAAUGUUGAAAG	1016	2899	GCAGUCAAAAUGUUGAAAG	1016	2921	CUUUCAACAUUUUGACUGC	1340
2917	GAAGGAGCAACACACAGUG	1017	2917	GAAGGAGCAACACACAGUG	1017	2939	CACUGUGUGUUGCUCCUUC	1341
2935	GAGCAUCGAGCUCUCAUGU	1018	2935	GAGCAUCGAGCUCUCAUGU	1018	2957	ACAUGAGAGCUCGAUGCUC	1342
2953	UCUGAACUCAAGAUCCUCA	1019	2953	UCUGAACUCAAGAUCCUCA	1019	2975	UGAGGAUCUUGAGUUCAGA	1343
2971	AUUCAUAUUGGUCACCAUC	1020	2971	AUUCAUAUUGGUCACCAUC	1020	2993	GAUGGUGACCAAUAUGAAU	1344
2989	CUCAAUGUGGUCAACCUUC	1021	2989	CUCAAUGUGGUCAACCUUC	1021	3011	GAAGGUUGACCACAUUGAG	1345
3007	CUAGGUGCCUGUACCAAGC	1022	3007	CUAGGUGCCUGUACCAAGC	1022	3029	GCUUGGUACAGGCACCUAG	1346
3025	CCAGGAGGGCCACUCAUGG	1023	3025	CCAGGAGGGCCACUCAUGG	1023	3047	CCAUGAGUGGCCCUCCUGG	1347
3043	GUGAUUGUGGAAUUCUGCA	1024	3043	GUGAUUGUGGAAUUCUGCA	1024	3065	UGCAGAAUUCCACAAUCAC	1348
3061	AAAUUUGGAAACCUGUCCA	1025	3061	AAAUUUGGAAACCUGUCCA	1025	3083	UGGACAGGUUUCCAAAUUU	1349
3079	ACUUACCUGAGGAGCAAGA	1026	3079	ACUUACCUGAGGAGCAAGA	1026	3101	UCUUGCUCCUCAGGUAAGU	1350
3097	AGAAAUGAAUUUGUCCCCU	1027	3097	AGAAAUGAAUUUGUCCCCU	1027	3119	AGGGGACAAAUUCAUUUCU	1351
3115	UACAAGACCAAAGGGGCAC	1028	3115	UACAAGACCAAAGGGGCAC	1028	3137	GUGCCCCUUUGGUCUUGUA	1352

3133	CGAUUCCGUCAAGGGAAAG	1029	3133	CGAUUCCGUCAAGGGAAAG	1029	3155	CUUUCCCUUGACGGAAUCG	1353
3151	GACUACGUUGGAGCAAUCC	1030	3151	GACUACGUUGGAGCAAUCC	1030	3173	GGAUUGCUCCAACGUAGUC	1354
3169	CCUGUGGAUCUGAAACGGC	1031	3169	CCUGUGGAUCUGAAACGGC	1031	3191	GCCGUUUCAGAUCCACAGG	1355
3187	CGCUUGGACAGCAUCACCA	1032	3187	CGCUUGGACAGCAUCACCA	1032	3209	UGGUGAUGCUGUCCAAGCG	1356
3205	AGUAGCCAGAGCUCAGCCA	1033	3205	AGUAGCCAGAGCUCAGCCA	1033	3227	UGGCUGAGCUCUGGCUACU	1357
3223	AGCUCUGGAUUUGUGGAGG	1034	3223	AGCUCUGGAUUUGUGGAGG	1034	3245	CCUCCACAAAUCCAGAGCU	1358
3241	GAGAAGUCCCUCAGUGAUG	1035	3241	GAGAAGUCCCUCAGUGAUG	1035	3263	CAUCACUGAGGGACUUCUC	1359
3259	GUAGAAGAAGAGGAAGCUC	1036	3259	GUAGAAGAAGAGGAAGCUC	1036	3281	GAGCUUCCUCUUCUUCUAC	1360
3277	CCUGAAGAUCUGUAUAAGG	1037	3277	CCUGAAGAUCUGUAUAAGG	1037	3299	CCUUAUACAGAUCUUCAGG	1361
3295	GACUUCCUGACCUUGGAGC	1038	3295	GACUUCCUGACCUUGGAGC	1038	3317	GCUCCAAGGUCAGGAAGUC	1362
3313	CAUCUCAUCUGUUACAGCU	1039	3313	CAUCUCAUCUGUUACAGCU	1039	3335	AGCUGUAACAGAUGAGAUG	1363
3331	UUCCAAGUGGCUAAGGGCA	1040	3331	UUCCAAGUGGCUAAGGGCA	1040	3353	UGCCCUUAGCCACUUGGAA	1364
3349	AUGGAGUUCUUGGCAUCGC	1041	3349	AUGGAGUUCUUGGCAUCGC	1041	3371	GCGAUGCCAAGAACUCCAU	1365
3367	CGAAAGUGUAUCCACAGGG	1042	3367	CGAAAGUGUAUCCACAGGG	1042	3389	CCCUGUGGAUACACUUUCG	1366
3385	GACCUGGCGGCACGAAAUA	1043	3385	GACCUGGCGGCACGAAAUA	1043	3407	UAUUUCGUGCCGCCAGGUC	1367
3403	AUCCUCUUAUCGGAGAAGA	1044	3403	AUCCUCUUAUCGGAGAAGA	1044	3425	UCUUCUCCGAUAAGAGGAU	1368
3421	AACGUGGUUAAAAUCUGUG	1045	3421	AACGUGGUUAAAAUCUGUG	1045	3443	CACAGAUUUUAACCACGUU	1369
3439	GACUUUGGCUUGGCCCGGG	1046	3439	GACUUUGGCUUGGCCCGGG	1046	3461	CCCGGGCCAAGCCAAAGUC	1370
3457	GAUAUUUAUAAAGAUCCAG	1047	3457	GAUAUUUAUAAAGAUCCAG	1047	3479	CUGGAUCUUUAUAAAUAUC	1371
3475	GAUUAUGUCAGAAAAGGAG	1048	3475	GAUUAUGUCAGAAAAGGAG	1048	3497	CUCCUUUUCUGACAUAAUC	1372
3493	GAUGCUCGCCUCCCUUUGA	1049	3493	GAUGCUCGCCUCCCUUUGA	1049	3515	UCAAAGGGAGGCGAGCAUC	1373
3511	AAAUGGAUGGCCCCAGAAA	1050	3511	AAAUGGAUGGCCCCAGAAA	1050	3533	UUUCUGGGGCCAUCCAUUU	1374
3529	ACAAUUUUUGACAGAGUGU	1051	3529	ACAAUUUUUGACAGAGUGU	1051	3551	ACACUCUGUCAAAAAUUGU	1375
3547	UACACAAUCCAGAGUGACG	1052	3547	UACACAAUCCAGAGUGACG	1052	3569	CGUCACUCUGGAUUGUGUA	1376
3565	GUCUGGUCUUUUGGUGUUU	1053	3565	GUCUGGUCUUUUGGUGUUU	1053	3587	AAACACCAAAAGACCAGAC	1377
3583	UUGCUGUGGGAAAUAUUUU	1054	3583	UUGCUGUGGGAAAUAUUUU	1054	3605	AAAAUAUUUCCCACAGCAA	1378
3601	UCCUUAGGUGCUUCUCCAU	1055	3601	UCCUUAGGUGCUUCUCCAU	1055	3623	AUGGAGAAGCACCUAAGGA	1379
3619	UAUCCUGGGGUAAAGAUUG	1056	3619	UAUCCUGGGGUAAAGAUUG	1056	3641	CAAUCUUUACCCCAGGAUA	1380
3637	GAUGAAGAAUUUUGUAGGC	1057	3637	GAUGAAGAAUUUUGUAGGC	1057	3659	GCCUACAAAAUUCUUCAUC	1381
3655	CGAUUGAAAGAAGGAACUA	1058	3655	CGAUUGAAAGAAGGAACUA	1058	3677	UAGUUCCUUCUUUCAAUCG	1382
3673	AGAAUGAGGGCCCCUGAUU	1059	3673	AGAAUGAGGGCCCCUGAUU	1059	3695	AAUCAGGGGCCCUCAUUCU	1383
3691	UAUACUACACCAGAAAUGU	1060	3691	UAUACUACACCAGAAAUGU	1060	3713	ACAUUUCUGGUGUAGUAUA	1384
3709	UACCAGACCAUGCUGGACU	1061	3709	UACCAGACCAUGCUGGACU	1061	3731	AGUCCAGCAUGGUCUGGUA	1385
3727	UGCUGGCACGGGGAGCCCA	1062	3727	UGCUGGCACGGGGAGCCCA	1062	3749	UGGGCUCCCCGUGCCAGCA	1386
3745	AGUCAGAGACCCACGUUUU	1063	3745	AGUCAGAGACCCACGUUUU	1063	3767	AAAACGUGGGUCUCUGACU	1387
3763	UCAGAGUUGGUGGAACAUU	1064	3763	UCAGAGUUGGUGGAACAUU	1064	3785	AAUGUUCCACCAACUCUGA	1388

3781	UUGGGAAAUCUCUUGCAAG	1065	3781	UUGGGAAAUCUCUUGCAAG	1065	3803	CUUGCAAGAGAUUUCCCAA	1389
3799	GCUAAUGCUCAGCAGGAUG	1066	3799	GCUAAUGCUCAGCAGGAUG	1066	3821	CAUCCUGCUGAGCAUUAGC	1390
3817	GGCAAAGACUACAUUGUUC	1067	3817	GGCAAAGACUACAUUGUUC	1067	3839	GAACAAUGUAGUCUUUGCC	1391
3835	CUUCCGAUAUCAGAGACUU	1068	3835	CUUCCGAUAUCAGAGACUU	1068	3857	AAGUCUCUGAUAUCGGAAG	1392
3853	UUGAGCAUGGAAGAGGAUU	1069	3853	UUGAGCAUGGAAGAGGAUU	1069	3875	AAUCCUCUUCCAUGCUCAA	1393
3871	UCUGGACUCUCUCUGCCUA	1070	3871	UCUGGACUCUCUCUGCCUA	1070	3893	UAGGCAGAGAGAGUCCAGA	1394
3889	ACCUCACCUGUUUCCUGUA	1071	3889	ACCUCACCUGUUUCCUGUA	1071	3911	UACAGGAAACAGGUGAGGU	1395
3907	AUGGAGGAGGAGGAAGUAU	1072	3907	AUGGAGGAGGAGGAAGUAU	1072	3929	AUACUUCCUCCUCCUCCAU	1396
3925	UGUGACCCCAAAUUCCAUU	1073	3925	UGUGACCCCAAAUUCCAUU	1073	3947	AAUGGAAUUUGGGGUCACA	1397
3943	UAUGACAACACAGCAGGAA	1074	3943	UAUGACAACACAGCAGGAA	1074	3965	UUCCUGCUGUGUUGUCAUA	1398
3961	AUCAGUCAGUAUCUGCAGA	1075	3961	AUCAGUCAGUAUCUGCAGA	1075	3983	UCUGCAGAUACUGACUGAU	1399
3979	AACAGUAAGCGAAAGAGCC	1076	3979	AACAGUAAGCGAAAGAGCC	1076	4001	GGCUCUUUCGCUUACUGUU	1400
3997	CGGCCUGUGAGUGUAAAAA	1077	3997	CGGCCUGUGAGUGUAAAAA	1077	4019	UUUUUACACUCACAGGCCG	1401
4015	ACAUUUGAAGAUAUCCCGU	1078	4015	ACAUUUGAAGAUAUCCCGU	1078	4037	ACGGGAUAUCUUCAAAUGU	1402
4033	UUAGAAGAACCAGAAGUAA	1079	4033	UUAGAAGAACCAGAAGUAA	1079	4055	UUACUUCUGGUUCUUCUAA	1403
4051	AAAGUAAUCCCAGAUGACA	1080	4051	AAAGUAAUCCCAGAUGACA	1080	4073	UGUCAUCUGGGAUUACUUU	1404
4069	AACCAGACGGACAGUGGUA	1081	4069	AACCAGACGGACAGUGGUA	1081	4091	UACCACUGUCCGUCUGGUU	1405
4087	AUGGUUCUUGCCUCAGAAG	1082	4087	AUGGUUCUUGCCUCAGAAG	1082	4109	CUUCUGAGGCAAGAACCAU	1406
4105	GAGCUGAAAACUUUGGAAG	1083	4105	GAGCUGAAAACUUUGGAAG	1083	4127	CUUCCAAAGUUUUCAGCUC	1407
4123	GACAGAACCAAAUUAUCUC	1084	4123	GACAGAACCAAAUUAUCUC	1084	4145	GAGAUAAUUUGGUUCUGUC	1408
4141	CCAUCUUUUGGUGGAAUGG	1085	4141	CCAUCUUUUGGUGGAAUGG	1085	4163	CCAUUCCACCAAAAGAUGG	1409
4159	GUGCCCAGCAAAAGCAGGG	1086	4159	GUGCCCAGCAAAAGCAGGG	1086	4181	CCCUGCUUUUGCUGGGCAC	1410
4177	GAGUCUGUGGCAUCUGAAG	1087	4177	GAGUCUGUGGCAUCUGAAG	1087	4199	CUUCAGAUGCCACAGACUC	1411
4195	GGCUCAAACCAGACAAGCG	1088	4195	GGCUCAAACCAGACAAGCG	1088	4217	CGCUUGUCUGGUUUGAGCC	1412
4213	GGCUACCAGUCCGGAUAUC	1089	4213	GGCUACCAGUCCGGAUAUC	1089	4235	GAUAUCCGGACUGGUAGCC	1413
4231	CACUCCGAUGACACAGACA	1090	4231	CACUCCGAUGACACAGACA	1090	4253	UGUCUGUGUCAUCGGAGUG	1414
4249	ACCACCGUGUACUCCAGUG	1091	4249	ACCACCGUGUACUCCAGUG	1091	4271	CACUGGAGUACACGGUGGU	1415
4267	GAGGAAGCAGAACUUUUAA	1092	4267	GAGGAAGCAGAACUUUUAA	1092	4289	UUAAAAGUUCUGCUUCCUC	1416
4285	AAGCUGAUAGAGAUUGGAG	1093	4285	AAGCUGAUAGAGAUUGGAG	1093	4307	CUCCAAUCUCUAUCAGCUU	1417
4303	GUGCAAACCGGUAGCACAG	1094	4303	GUGCAAACCGGUAGCACAG	1094	4325	CUGUGCUACCGGUUUGCAC	1418
4321	GCCCAGAUUCUCCAGCCUG	1095	4321	GCCCAGAUUCUCCAGCCUG	1095	4343	CAGGCUGGAGAAUCUGGGC	1419
4339	GACUCGGGGACCACACUGA	1096	4339	GACUCGGGGACCACACUGA	1096	4361	UCAGUGUGGUCCCCGAGUC	1420
4357	AGCUCUCCUCCUGUUUAAA	1097	4357	AGCUCUCCUCCUGUUUAAA	1097	4379	UUUAAACAGGAGGAGAGCU	1421
4375	AAGGAAGCAUCCACACCCC	1098	4375	AAGGAAGCAUCCACACCCC	1098	4397	GGGGUGUGGAUGCUUCCUU	1422
4393	CAACUCCCGGACAUCACAU	1099	4393	CAACUCCCGGACAUCACAU	1099	4415	AUGUGAUGUCCGGGAGUUG	1423
4411	UGAGAGGUCUGCUCAGAUU	1100	4411	UGAGAGGUCUGCUCAGAUU	1100	4433	AAUCUGAGCAGACCUCUCA	1424

4429	UUUGAAGUGUUGUUCUUUC	1101	4429	UUUGAAGUGUUGUUCUUUC	1101	4451	GAAAGAACAACACUUCAAA	1425
4447	CCACCAGCAGGAAGUAGCC	1102	4447	CCACCAGCAGGAAGUAGCC	1102	4469	GGCUACUUCCUGCUGGUGG	1426
4465	CGCAUUUGAUUUUCAUUUC	1103	4465	CGCAUUUGAUUUUCAUUUC	1103	4487	GAAAUGAAAAUCAAAUGCG	1427
4483	CGACAACAGAAAAAGGACC	1104	4483	CGACAACAGAAAAAGGACC	1104	4505	GGUCCUUUUUCUGUUGUCG	1428
4501	CUCGGACUGCAGGGAGCCA	1105	4501	CUCGGACUGCAGGGAGCCA	1105	4523	UGGCUCCCUGCAGUCCGAG	1429
4519	AGUCUUCUAGGCAUAUCCU	1106	4519	AGUCUUCUAGGCAUAUCCU	1106	4541	AGGAUAUGCCUAGAAGACU	1430
4537	UGGAAGAGGCUUGUGACCC	1107	4537	UGGAAGAGGCUUGUGACCC	1107	4559	GGGUCACAAGCCUCUUCCA	1431
4555	CAAGAAUGUGUCUGUGUCU	1108	4555	CAAGAAUGUGUCUGUGUCU	1108	4577	AGACACAGACACAUUCUUG	1432
4573	UUCUCCCAGUGUUGACCUG	1109	4573	UUCUCCCAGUGUUGACCUG	1109	4595	CAGGUCAACACUGGGAGAA	1433
4591	GAUCCUCUUUUUUCAUUCA	1110	4591	GAUCCUCUUUUUUCAUUCA	1110	4613	UGAAUGAAAAAAGAGGAUC	1434
4609	AUUUAAAAAGCAUUAUCAU	1111	4609	AUUUAAAAAGCAUUAUCAU	1111	4631	AUGAUAAUGCUUUUUAAAU	1435
4627	UGCCCCUGCUGCGGGUCUC	1112	4627	UGCCCCUGCUGCGGGUCUC	1112	4649	GAGACCCGCAGCAGGGGCA	1436
4645	CACCAUGGGUUUAGAACAA	1113	4645	CACCAUGGGUUUAGAACAA	1113	4667	UUGUUCUAAACCCAUGGUG	1437
4663	AAGAGCUUCAAGCAAUGGC	1114	4663	AAGAGCUUCAAGCAAUGGC	1114	4685	GCCAUUGCUUGAAGCUCUU	1438
4681	CCCCAUCCUCAAAGAAGUA	1115	4681	CCCCAUCCUCAAAGAAGUA	1115	4703	UACUUCUUUGAGGAUGGGG	1439
4699	AGCAGUACCUGGGGAGCUG	1116	4699	AGCAGUACCUGGGGAGCUG	1116	4721	CAGCUCCCCAGGUACUGCU	1440
4717	GACACUUCUGUAAAACUAG	1117	4717	GACACUUCUGUAAAACUAG	1117	4739	CUAGUUUUACAGAAGUGUC	1441
4735	GAAGAUAAACCAGGCAACG	1118	4735	GAAGAUAAACCAGGCAACG	1118	4757	CGUUGCCUGGUUUAUCUUC	1442
4753	GUAAGUGUUCGAGGUGUUG	1119	4753	GUAAGUGUUCGAGGUGUUG	1119	4775	CAACACCUCGAACACUUAC	1443
4771	GAAGAUGGGAAGGAUUUGC	1120	4771	GAAGAUGGGAAGGAUUUGC	1120	4793	GCAAAUCCUUCCCAUCUUC	1444
4789	CAGGGCUGAGUCUAUCCAA	1121	4789	CAGGGCUGAGUCUAUCCAA	1121	4811	UUGGAUAGACUCAGCCCUG	1445
4807	AGAGGCUUUGUUUAGGACG	1122	4807	AGAGGCUUUGUUUAGGACG	1122	4829	CGUCCUAAACAAAGCCUCU	1446
4825	GUGGGUCCCAAGCCAAGCC	1123	4825	GUGGGUCCCAAGCCAAGCC	1123	4847	GGCUUGGCUUGGGACCCAC	1447
4843	CUUAAGUGUGGAAUUCGGA	1124	4843	CUUAAGUGUGGAAUUCGGA	1124	4865	UCCGAAUUCCACACUUAAG	1448
4861	AUUGAUAGAAAGGAAGACU	1125	4861	AUUGAUAGAAAGGAAGACU	1125	4883	AGUCUUCCUUUCUAUCAAU	1449
4879	UAACGUUACCUUGCUUUGG	1126	4879	UAACGUUACCUUGCUUUGG	1126	4901	CCAAAGCAAGGUAACGUUA	1450
4897	GAGAGUACUGGAGCCUGCA	1127	4897	GAGAGUACUGGAGCCUGCA	1127	4919	UGCAGGCUCCAGUACUCUC	1451
4915	AAAUGCAUUGUGUUUGCUC	1128	4915	AAAUGCAUUGUGUUUGCUC	1128	4937	GAGCAAACACAAUGCAUUU	1452
4933	CUGGUGGAGGUGGGCAUGG	1129	4933	CUGGUGGAGGUGGGCAUGG	1129	4955	CCAUGCCCACCUCCACCAG	1453
4951	GGGUCUGUUCUGAAAUGUA	1130	4951	GGGUCUGUUCUGAAAUGUA	1130	4973	UACAUUUCAGAACAGACCC	1454
4969	AAAGGGUUCAGACGGGGUU	1131	4969	AAAGGGUUCAGACGGGGUU	1131	4991	AACCCCGUCUGAACCCUUU	1455
4987	UUCUGGUUUUAGAAGGUUG	1132	4987	UUCUGGUUUUAGAAGGUUG	1132	5009	CAACCUUCUAAAACCAGAA	1456
5005	GCGUGUUCUUCGAGUUGGG	1133	5005	GCGUGUUCUUCGAGUUGGG	1133	5027	CCCAACUCGAAGAACACGC	1457
5023	GCUAAAGUAGAGUUCGUUG	1134	5023	GCUAAAGUAGAGUUCGUUG	1134	5045	CAACGAACUCUACUUUAGC	1458
5041	GUGCUGUUUCUGACUCCUA	1135	5041	GUGCUGUUUCUGACUCCUA	1135	5063	UAGGAGUCAGAAACAGCAC	1459
5059	AAUGAGAGUUCCUUCCAGA	1136	5059	AAUGAGAGUUCCUUCCAGA	1136	5081	UCUGGAAGGAACUCUCAUU	1460

5077	ACCGUUAGCUGUCUCCUUG	1137	5077	ACCGUUAGCUGUCUCCUUG	1137	5099	CAAGGAGACAGCUAACGGU	1461
5095	GCCAAGCCCCAGGAAGAAA	1138	5095	GCCAAGCCCCAGGAAGAAA	1138	5117	UUUCUUCCUGGGGCUUGGC	1462
5113	AAUGAUGCAGCUCUGGCUC	1139	5113	AAUGAUGCAGCUCUGGCUC	1139	5135	GAGCCAGAGCUGCAUCAUU	1463
5131	CCUUGUCUCCCAGGCUGAU	1140	5131	CCUUGUCUCCCAGGCUGAU	1140	5153	AUCAGCCUGGGAGACAAGG	1464
5149	UCCUUUAUUCAGAAUACCA	1141	5149	UCCUUUAUUCAGAAUACCA	1141	5171	UGGUAUUCUGAAUAAAGGA	1465
5167	ACAAAGAAAGGACAUUCAG	1142	5167	ACAAAGAAAGGACAUUCAG	1142	5189	CUGAAUGUCCUUUCUUUGU	1466
5185	GCUCAAGGCUCCCUGCCGU	1143	5185	GCUCAAGGCUCCCUGCCGU	1143	5207	ACGGCAGGGAGCCUUGAGC	1467
5203	UGUUGAAGAGUUCUGACUG	1144	5203	UGUUGAAGAGUUCUGACUG	1144	5225	CAGUCAGAACUCUUCAACA	1468
5221	GCACAAACCAGCUUCUGGU	1145	5221	GCACAAACCAGCUUCUGGU	1145	5243	ACCAGAAGCUGGUUUGUGC	1469
5239	UUUCUUCUGGAAUGAAUAC	1146	5239	UUUCUUCUGGAAUGAAUAC	1146	5261	GUAUUCAUUCCAGAAGAAA	1470
5257	CCCUCAUAUCUGUCCUGAU	1147	5257	CCCUCAUAUCUGUCCUGAU	1147	5279	AUCAGGACAGAUAUGAGGG	1471
5275	UGUGAUAUGUCUGAGACUG	1148	5275	UGUGAUAUGUCUGAGACUG	1148	5297	CAGUCUCAGACAUAUCACA	1472
5293	GAAUGCGGGAGGUUCAAUG	1149	5293	GAAUGCGGGAGGUUCAAUG	1149	5315	CAUUGAACCUCCCGCAUUC	1473
5311	GUGAAGCUGUGUGUGGUGU	1150	5311	GUGAAGCUGUGUGUGGUGU	1150	5333	ACACCACACACAGCUUCAC	1474
5329	UCAAAGUUUCAGGAAGGAU	1151	5329	UCAAAGUUUCAGGAAGGAU	1151	5351	AUCCUUCCUGAAACUUUGA	1475
5347	UUUUACCCUUUUGUUCUUC	1152	5347	UUUUACCCUUUUGUUCUUC	1152	5369	GAAGAACAAAAGGGUAAAA	1476
5365	CCCCCUGUCCCCAACCCAC	1153	5365	CCCCCUGUCCCCAACCCAC	1153	5387	GUGGGUUGGGGACAGGGGG	1477
5383	CUCUCACCCCGCAACCCAU	1154	5383	CUCUCACCCCGCAACCCAU	1154	5405	AUGGGUUGCGGGGUGAGAG	1478
5401	UCAGUAUUUUAGUUAUUUG	1155	5401	UCAGUAUUUUAGUUAUUUG	1155	5423	CAAAUAACUAAAAUACUGA	1479
5419	GGCCUCUACUCCAGUAAAC	1156	5419	GGCCUCUACUCCAGUAAAC	1156	5441	GUUUACUGGAGUAGAGGCC	1480
5437	CCUGAUUGGGUUUGUUCAC	1157	5437	CCUGAUUGGGUUUGUUCAC	1157	5459	GUGAACAAACCCAAUCAGG	1481
5455	CUCUCUGAAUGAUUAUUAG	1158	5455	CUCUCUGAAUGAUUAUUAG	1158	5477	CUAAUAAUCAUUCAGAGAG	1482
5473	GCCAGACUUCAAAAUUAUU	1159	5473	GCCAGACUUCAAAAUUAUU	1159	5495	AAUAAUUUUGAAGUCUGGC	1483
5491	UUUAUAGCCCAAAUUAUAA	1160	5491	UUUAUAGCCCAAAUUAUAA	1160	5513	UUAUAAUUUGGGCUAUAAA	1484
5509	ACAUCUAUUGUAUUAUUUA	1161	5509	ACAUCUAUUGUAUUAUUUA	1161	5531	UAAAUAAUACAAUAGAUGU	1485
5527	AGACUUUUAACAUAUAGAG	1162	5527	AGACUUUUAACAUAUAGAG	1162	5549	CUCUAUAUGUUAAAAGUCU	1486
5545	GCUAUUUCUACUGAUUUUU	1163	5545	GCUAUUUCUACUGAUUUUU	1163	5567	AAAAAUCAGUAGAAAUAGC	1487
5563	UGCCCUUGUUCUGUCCUUU	1164	5563	UGCCCUUGUUCUGUCCUUU	1164	5585	AAAGGACAGAACAAGGGCA	1488
5581	UUUUUCAAAAAAGAAAAUG	1165	5581	UUUUUCAAAAAAGAAAAUG	1165	5603	CAUUUUCUUUUUUGAAAAA	1489
5599	GUGUUUUUUGUUUGGUACC	1166	5599	GUGUUUUUUGUUUGGUACC	1166	5621	GGUACCAAACAAAAAACAC	1490
5617	CAUAGUGUGAAAUGCUGGG	1167	5617	CAUAGUGUGAAAUGCUGGG	1167	5639	CCCAGCAUUUCACACUAUG	1491
5635	GAACAAUGACUAUAAGACA	1168	5635	GAACAAUGACUAUAAGACA	1168	5657	UGUCUUAUAGUCAUUGUUC	1492
5653	AUGCUAUGGCACAUAUAUU	1169	5653	AUGCUAUGGCACAUAUAUU	1169	5675	AAUAUAUGUGCCAUAGCAU	1493
5671	UUAUAGUCUGUUUAUGUAG	1170	5671	UUAUAGUCUGUUUAUGUAG	1170	5693	CUACAUAAACAGACUAUAA	1494
5689	GAAACAAAUGUAAUAUAUU	1171	5689	GAAACAAAUGUAAUAUAUU	1171	5711	AAUAUAUUACAUUUGUUUC	1495
5707	UAAAGCCUUAUAUAUAAUG	1172	5707	UAAAGCCUUAUAUAUAAUG	1172	5729	CAUUAUAUAUAAGGCUUUA	1496

5725	GAACUUUGUACUAUUCACA	1173	5725	GAACUUUGUACUAUUCACA	1173	5747	UGUGAAUAGUACAAAGUUC	1497
5743	AUUUUGUAUCAGUAUUAUG	1174	5743	AUUUUGUAUCAGUAUUAUG	1174	5765	CAUAAUACUGAUACAAAAU	1498
5761	GUAGCAUAACAAAGGUCAU	1175	5761	GUAGCAUAACAAAGGUCAU	1175	5783	AUGACCUUUGUUAUGCUAC	1499
5779	UAAUGCUUUCAGCAAUUGA	1176	5779	UAAUGCUUUCAGCAAUUGA	1176	5801	UCAAUUGCUGAAAGCAUUA	1500
5797	AUGUCAUUUUAUUAAAGAA	1177	5797	AUGUCAUUUUAUUAAAGAA	1177	5819	UUCUUUAAUAAAAUGACAU	1501
5812	AGAACAUUGAAAAACUUGA	1178	5812	AGAACAUUGAAAAACUUGA	1178	5834	UCAAGUUUUUCAAUGUUCU	1502

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Pos	Target Sequence	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
1	ACCCACGCGCAGCGGCCGG	1503	1	ACCCACGCGCAGCGGCCGG	1503	23	CCGGCCGCUGCGCGUGGGU	1750
19	GAGAUGCAGCGGGGCGCCG	1504	19	GAGAUGCAGCGGGGCGCCG	1504	41	CGGCGCCCCGCUGCAUCUC	1751
37	GCGCUGUGCCUGCGACUGU	1505	37	GCGCUGUGCCUGCGACUGU	1505	59	ACAGUCGCAGGCACAGCGC	1752
55	UGGCUCUGCCUGGGACUCC	1506	55	UGGCUCUGCCUGGGACUCC	1506	77	GGAGUCCCAGGCAGAGCCA	1753
73	CUGGACGGCCUGGUGAGUG	1507	73	CUGGACGGCCUGGUGAGUG	1507	95	CACUCACCAGGCCGUCCAG	1754
91	GACUACUCCAUGACCCCCC	1508	91	GACUACUCCAUGACCCCCC	1508	113	GGGGGGUCAUGGAGUAGUC	1755
109	CCGACCUUGAACAUCACGG	1509	109	CCGACCUUGAACAUCACGG	1509	131	CCGUGAUGUUCAAGGUCGG	1756
127	GAGGAGUCACACGUCAUCG	1510	127	GAGGAGUCACACGUCAUCG	1510	149	CGAUGACGUGUGACUCCUC	1757
145	GACACCGGUGACAGCCUGU	1511	145	GACACCGGUGACAGCCUGU	1511	167	ACAGGCUGUCACCGGUGUC	1758
163	UCCAUCUCCUGCAGGGGAC	1512	163	UCCAUCUCCUGCAGGGGAC	1512	185	GUCCCCUGCAGGAGAUGGA	1759
181	CAGCACCCCCUCGAGUGGG	1513	181	CAGCACCCCCUCGAGUGGG	1513	203	CCCACUCGAGGGGGUGCUG	1760
199	GCUUGGCCAGGAGCUCAGG	1514	199	GCUUGGCCAGGAGCUCAGG	1514	221	CCUGAGCUCCUGGCCAAGC	1761
217	GAGGCGCCAGCCACCGGAG	1515	217	GAGGCGCCAGCCACCGGAG	1515	239	CUCCGGUGGCUGGCGCCUC	1762
235	GACAAGGACAGCGAGGACA	1516	235	GACAAGGACAGCGAGGACA	1516	257	UGUCCUCGCUGUCCUUGUC	1763
253	ACGGGGGUGGUGCGAGACU	1517	253	ACGGGGGUGGUGCGAGACU	1517	275	AGUCUCGCACCACCCCCGU	1764
271	UGCGAGGGCACAGACGCCA	1518	271	UGCGAGGGCACAGACGCCA	1518	293	UGGCGUCUGUGCCCUCGCA	1765
289	AGGCCCUACUGCAAGGUGU	1519	289	AGGCCCUACUGCAAGGUGU	1519	311	ACACCUUGCAGUAGGGCCU	1766
307	UUGCUGCUGCACGAGGUAC	1520	307	UUGCUGCUGCACGAGGUAC	1520	329	GUACCUCGUGCAGCAGCAA	1767
325	CAUGCCAACGACACAGGCA	1521	325	CAUGCCAACGACACAGGCA	1521	347	UGCCUGUGUCGUUGGCAUG	1768
343	AGCUACGUCUGCUACUACA	1522	343	AGCUACGUCUGCUACUACA	1522	365	UGUAGUAGCAGACGUAGCU	1769
361	AAGUACAUCAAGGCACGCA	1523	361	AAGUACAUCAAGGCACGCA	1523	383	UGCGUGCCUUGAUGUACUU	1770
379	AUCGAGGGCACCACGGCCG	1524	379	AUCGAGGGCACCACGGCCG	1524	401	CGGCCGUGGUGCCCUCGAU	1771
397	GCCAGCUCCUACGUGUUCG	1525	397	GCCAGCUCCUACGUGUUCG	1525	419	CGAACACGUAGGAGCUGGC	1772

415	GUGAGAGACUUUGAGCAGC	1526	415	GUGAGAGACUUUGAGCAGC	1526	437	GCUGCUCAAAGUCUCUCAC	1773
433	CCAUUCAUCAACAAGCCUG	1527	433	CCAUUCAUCAACAAGCCUG	1527	455	CAGGCUUGUUGAUGAAUGG	1774
451	GACACGCUCUUGGUCAACA	1528	451	GACACGCUCUUGGUCAACA	1528	473	UGUUGACCAAGAGCGUGUC	1775
469	AGGAAGGACGCCAUGUGGG	1529	469	AGGAAGGACGCCAUGUGGG	1529	491	CCCACAUGGCGUCCUUCCU	1776
487	GUGCCCUGUCUGGUGUCCA	1530	487	GUGCCCUGUCUGGUGUCCA	1530	509	UGGACACCAGACAGGGCAC	1777
505	AUCCCCGGCCUCAAUGUCA	1531	505	AUCCCCGGCCUCAAUGUCA	1531	527	UGACAUUGAGGCCGGGGAU	1778
523	ACGCUGCGCUCGCAAAGCU	1532	523	ACGCUGCGCUCGCAAAGCU	1532	545	AGCUUUGCGAGCGCAGCGU	1779
541	UCGGUGCUGUGGCCAGACG	1533	541	UCGGUGCUGUGGCCAGACG	1533	563	CGUCUGGCCACAGCACCGA	1780
559	GGGCAGGAGGUGGUGUGGG	1534	559	GGGCAGGAGGUGGUGUGGG	1534	581	CCCACACCACCUCCUGCCC	1781
577	GAUGACCGGCGGGGCAUGC	1535	577	GAUGACCGGCGGGGCAUGC	1535	599	GCAUGCCCCGCCGGUCAUC	1782
595	CUCGUGUCCACGCCACUGC	1536	595	CUCGUGUCCACGCCACUGC	1536	617	GCAGUGGCGUGGACACGAG	1783
613	CUGCACGAUGCCCUGUACC	1537	613	CUGCACGAUGCCCUGUACC	1537	635	GGUACAGGGCAUCGUGCAG	1784
631	CUGCAGUGCGAGACCACCU	1538	631	CUGCAGUGCGAGACCACCU	1538	653	AGGUGGUCUCGCACUGCAG	1785
649	UGGGGAGACCAGGACUUCC	1539	649	UGGGGAGACCAGGACUUCC	1539	671	GGAAGUCCUGGUCUCCCCA	1786
667	CUUUCCAACCCCUUCCUGG	1540	667	CUUUCCAACCCCUUCCUGG	1540	689	CCAGGAAGGGGUUGGAAAG	1787
685	GUGCACAUCACAGGCAACG	1541	685	GUGCACAUCACAGGCAACG	1541	707	CGUUGCCUGUGAUGUGCAC	1788
703	GAGCUCUAUGACAUCCAGC	1542	703	GAGCUCUAUGACAUCCAGC	1542	725	GCUGGAUGUCAUAGAGCUC	1789
721	CUGUUGCCCAGGAAGUCGC	1543	721	CUGUUGCCCAGGAAGUCGC	1543	743	GCGACUUCCUGGGCAACAG	1790
739	CUGGAGCUGCUGGUAGGGG	1544	739	CUGGAGCUGCUGGUAGGGG	1544	761	CCCCUACCAGCAGCUCCAG	1791
757	GAGAAGCUGGUCCUCAACU	1545	757	GAGAAGCUGGUCCUCAACU	1545	779	AGUUGAGGACCAGCUUCUC	1792
775	UGCACCGUGUGGGCUGAGU	1546	775	UGCACCGUGUGGGCUGAGU	1546	797	ACUCAGCCCACACGGUGCA	1793
793	UUUAACUCAGGUGUCACCU	1547	793	UUUAACUCAGGUGUCACCU	1547	815	AGGUGACACCUGAGUUAAA	1794
811	UUUGACUGGGACUACCCAG	1548	811	UUUGACUGGGACUACCCAG	1548	833	CUGGGUAGUCCCAGUCAAA	1795
829	GGGAAGCAGGCAGAGCGGG	1549	829	GGGAAGCAGGCAGAGCGGG	1549	851	CCCGCUCUGCCUGCUUCCC	1796
847	GGUAAGUGGGUGCCCGAGC	1550	847	GGUAAGUGGGUGCCCGAGC	1550	869	GCUCGGGCACCCACUUACC	1797
865	CGACGCUCCCAACAGACCC	1551	865	CGACGCUCCCAACAGACCC	1551	887	GGGUCUGUUGGGAGCGUCG	1798
883	CACACAGAACUCUCCAGCA	1552	883	CACACAGAACUCUCCAGCA	1552	905	UGCUGGAGAGUUCUGUGUG	1799
901	AUCCUGACCAUCCACAACG	1553	901	AUCCUGACCAUCCACAACG	1553	923	CGUUGUGGAUGGUCAGGAU	1800
919	GUCAGCCAGCACGACCUGG	1554	919	GUCAGCCAGCACGACCUGG	1554	941	CCAGGUCGUGCUGGCUGAC	1801
937	GGCUCGUAUGUGUGCAAGG	1555	937	GGCUCGUAUGUGUGCAAGG	1555	959	CCUUGCACACAUACGAGCC	1802
955	GCCAACAACGGCAUCCAGC	1556	955	GCCAACAACGGCAUCCAGC	1556	977	GCUGGAUGCCGUUGUUGGC	1803
973	CGAUUUCGGGAGAGCACCG	1557	973	CGAUUUCGGGAGAGCACCG	1557	995	CGGUGCUCUCCCGAAAUCG	1804
991	GAGGUCAUUGUGCAUGAAA	1558	991	GAGGUCAUUGUGCAUGAAA	1558	1013	UUUCAUGCACAAUGACCUC	1805

1009	AAUCCCUUCAUCAGCGUCG	1559	1009	AAUCCCUUCAUCAGCGUCG	1559	1031	CGACGCUGAUGAAGGGAUU	1806
1027	GAGUGGCUCAAAGGACCCA	1560	1027	GAGUGGCUCAAAGGACCCA	1560	1049	UGGGUCCUUUGAGCCACUC	1807
1045	AUCCUGGAGGCCACGGCAG	1561	1045	AUCCUGGAGGCCACGGCAG	1561	1067	CUGCCGUGGCCUCCAGGAU	1808
1063	GGAGACGAGCUGGUGAAGC	1562	1063	GGAGACGAGCUGGUGAAGC	1562	1085	GCUUCACCAGCUCGUCUCC	1809
1081	CUGCCCGUGAAGCUGGCAG	1563	1081	CUGCCCGUGAAGCUGGCAG	1563	1103	CUGCCAGCUUCACGGGCAG	1810
1099	GCGUACCCCCCGCCCGAGU	1564	1099	GCGUACCCCCCGCCCGAGU	1564	1121	ACUCGGGCGGGGGGUACGC	1811
1117	UUCCAGUGGUACAAGGAUG	1565	1117	UUCCAGUGGUACAAGGAUG	1565	1139	CAUCCUUGUACCACUGGAA	1812
1135	GGAAAGGCACUGUCCGGGC	1566	1135	GGAAAGGCACUGUCCGGGC	1566	1157	GCCCGGACAGUGCCUUUCC	1813
1153	CGCCACAGUCCACAUGCCC	1567	1153	CGCCACAGUCCACAUGCCC	1567	1175	GGGCAUGUGGACUGUGGCG	1814
1171	CUGGUGCUCAAGGAGGUGA	1568	1171	CUGGUGCUCAAGGAGGUGA	1568	1193	UCACCUCCUUGAGCACCAG	1815
1189	ACAGAGGCCAGCACAGGCA	1569	1189	ACAGAGGCCAGCACAGGCA	1569	1211	UGCCUGUGCUGGCCUCUGU	1816
1207	ACCUACACCCUCGCCCUGU	1570	1207	ACCUACACCCUCGCCCUGU	1570	1229	ACAGGGCGAGGGUGUAGGU	1817
1225	UGGAACUCCGCUGCUGGCC	1571	1225	UGGAACUCCGCUGCUGGCC	1571	1247	GGCCAGCAGCGGAGUUCCA	1818
1243	CUGAGGCGCAACAUCAGCC	1572	1243	CUGAGGCGCAACAUCAGCC	1572	1265	GGCUGAUGUUGCGCCUCAG	1819
1261	CUGGAGCUGGUGGUGAAUG	1573	1261	CUGGAGCUGGUGGUGAAUG	1573	1283	CAUUCACCACCAGCUCCAG	1820
1279	GUGCCCCCCAGAUACAUG	1574	1279	GUGCCCCCCAGAUACAUG	1574	1301	CAUGUAUCUGGGGGGCAC	1821
1297	GAGAAGGAGGCCUCCUCCC	1575	1297	GAGAAGGAGGCCUCCUCCC	1575	1319	GGGAGGAGGCCUCCUUCUC	1822
1315	CCCAGCAUCUACUCGCGUC	1576	1315	CCCAGCAUCUACUCGCGUC	1576	1337	GACGCGAGUAGAUGCUGGG	1823
1333	CACAGCCGCCAGGCCCUCA	1577	1333	CACAGCCGCCAGGCCCUCA	1577	1355	UGAGGGCCUGGCGGCUGUG	1824
1351	ACCUGCACGGCCUACGGGG	1578	1351	ACCUGCACGGCCUACGGGG	1578	1373	CCCCGUAGGCCGUGCAGGU	1825
1369	GUGCCCCUGCCUCUCAGCA	1579	1369	GUGCCCCUGCCUCUCAGCA	1579	1391	UGCUGAGAGGCAGGGGCAC	1826
1387	AUCCAGUGGCACUGGCGGC	1580	1387	AUCCAGUGGCACUGGCGGC	1580	1409	GCCGCCAGUGCCACUGGAU	1827
1405	CCCUGGACACCCUGCAAGA	1581	1405	CCCUGGACACCCUGCAAGA	1581	1427	UCUUGCAGGGUGUCCAGGG	1828
1423	AUGUUUGCCCAGCGUAGUC	1582	1423	AUGUUUGCCCAGCGUAGUC	1582	1445	GACUACGCUGGGCAAACAU	1829
1441	CUCCGGCGGCGGCAGCAGC	1583	1441	CUCCGGCGGCGGCAGCAGC	1583	1463	GCUGCUGCCGCCGCCGGAG	1830
1459	CAAGACCUCAUGCCACAGU	1584	1459	CAAGACCUCAUGCCACAGU	1584	1481	ACUGUGGCAUGAGGUCUUG	1831
1477	UGCCGUGACUGGAGGGCGG	1585	1477	UGCCGUGACUGGAGGGCGG	1585	1499	CCGCCCUCCAGUCACGGCA	1832
1495	GUGACCACGCAGGAUGCCG	1586	1495	GUGACCACGCAGGAUGCCG	1586	1517	CGGCAUCCUGCGUGGUCAC	1833
1513	GUGAACCCCAUCGAGAGCC	1587	1513	GUGAACCCCAUCGAGAGCC	1587	1535	GGCUCUCGAUGGGGUUCAC	1834
1531	CUGGACACCUGGACCGAGU	1588	1531	CUGGACACCUGGACCGAGU	1588	1553	ACUCGGUCCAGGUGUCCAG	1835
1549	UUUGUGGAGGGAAAGAAUA	1589	1549	UUUGUGGAGGGAAAGAAUA	1589	1571	UAUUCUUUCCCUCCACAAA	1836
1567	AAGACUGUGAGCAAGCUGG	1590	1567	AAGACUGUGAGCAAGCUGG	1590	1589	CCAGCUUGCUCACAGUCUU	1837
1585	GUGAUCCAGAAUGCCAACG	1591	1585	GUGAUCCAGAAUGCCAACG	1591	1607	CGUUGGCAUUCUGGAUCAC	1838

1603	GUGUCUGCCAUGUACAAGU	1592	1603	GUGUCUGCCAUGUACAAGU	1592	1625	ACUUGUACAUGGCAGACAC	1839
1621	UGUGUGGUCUCCAACAAGG	1593	1621	UGUGUGGUCUCCAACAAGG	1593	1643	CCUUGUUGGAGACCACACA	1840
1639	GUGGGCCAGGAUGAGCGGC	1594	1639	GUGGGCCAGGAUGAGCGGC	1594	1661	GCCGCUCAUCCUGGCCCAC	1841
1657	CUCAUCUACUUCUAUGUGA	1595	1657	CUCAUCUACUUCUAUGUGA	1595	1679	UCACAUAGAAGUAGAUGAG	1842
1675	ACCACCAUCCCCGACGGCU	1596	1675	ACCACCAUCCCCGACGGCU	1596	1697	AGCCGUCGGGGAUGGUGGU	1843
1693	UUCACCAUCGAAUCCAAGC	1597	1693	UUCACCAUCGAAUCCAAGC	1597	1715	GCUUGGAUUCGAUGGUGAA	1844
1711	CCAUCCGAGGAGCUACUAG	1598	1711	CCAUCCGAGGAGCUACUAG	1598	1733	CUAGUAGCUCCUCGGAUGG	1845
1729	GAGGGCCAGCCGGUGCUCC	1599	1729	GAGGGCCAGCCGGUGCUCC	1599	1751	GGAGCACCGGCUGGCCCUC	1846
1747	CUGAGCUGCCAAGCCGACA	1600	1747	CUGAGCUGCCAAGCCGACA	1600	1769	UGUCGGCUUGGCAGCUCAG	1847
1765	AGCUACAAGUACGAGCAUC	1601	1765	AGCUACAAGUACGAGCAUC	1601	1787	GAUGCUCGUACUUGUAGCU	1848
1783	CUGCGCUGGUACCGCCUCA	1602	1783	CUGCGCUGGUACCGCCUCA	1602	1805	UGAGGCGGUACCAGCGCAG	1849
1801	AACCUGUCCACGCUGCACG	1603	1801	AACCUGUCCACGCUGCACG	1603	1823	CGUGCAGCGUGGACAGGUU	1850
1819	GAUGCGCACGGGAACCCGC	1604	1819	GAUGCGCACGGGAACCCGC	1604	1841	GCGGGUUCCCGUGCGCAUC	1851
1837	CUUCUGCUCGACUGCAAGA	1605	1837	CUUCUGCUCGACUGCAAGA	1605	1859	UCUUGCAGUCGAGCAGAAG	1852
1855	AACGUGCAUCUGUUCGCCA	1606	1855	AACGUGCAUCUGUUCGCCA	1606	1877	UGGCGAACAGAUGCACGUU	1853
1873	ACCCCUCUGGCCGCCAGCC	1607	1873	ACCCCUCUGGCCGCCAGCC	1607	1895	GGCUGGCGGCCAGAGGGGU	1854
1891	CUGGAGGAGGUGGCACCUG	1608	1891	CUGGAGGAGGUGGCACCUG	1608	1913	CAGGUGCCACCUCCUCCAG	1855
1909	GGGGCGCGCCACGCCACGC	1609	1909	GGGGCGCGCCACGCCACGC	1609	1931	GCGUGGCGUGGCGCGCCCC	1856
1927	CUCAGCCUGAGUAUCCCCC	1610	1927	CUCAGCCUGAGUAUCCCCC	1610	1949	GGGGGAUACUCAGGCUGAG	1857
1945	CGCGUCGCGCCCGAGCACG	1611	1945	CGCGUCGCGCCCGAGCACG	1611	1967	CGUGCUCGGGCGCGACGCG	1858
1963	GAGGGCCACUAUGUGUGCG	1612	1963	GAGGGCCACUAUGUGUGCG	1612	1985	CGCACACAUAGUGGCCCUC	1859
1981	GAAGUGCAAGACCGGCGCA	1613	1981	GAAGUGCAAGACCGGCGCA	1613	2003	UGCGCCGGUCUUGCACUUC	1860
1999	AGCCAUGACAAGCACUGCC	1614	1999	AGCCAUGACAAGCACUGCC	1614	2021	GGCAGUGCUUGUCAUGGCU	1861
2017	CACAAGAAGUACCUGUCGG	1615	2017	CACAAGAAGUACCUGUCGG	1615	2039	CCGACAGGUACUUCUUGUG	1862
2035	GUGCAGGCCCUGGAAGCCC	1616	2035	GUGCAGGCCCUGGAAGCCC	1616	2057	GGGCUUCCAGGGCCUGCAC	1863
2053	CCUCGGCUCACGCAGAACU	1617	2053	CCUCGGCUCACGCAGAACU	1617	2075	AGUUCUGCGUGAGCCGAGG	1864
2071	UUGACCGACCUCCUGGUGA	1618	2071	UUGACCGACCUCCUGGUGA	1618	2093	UCACCAGGAGGUCGGUCAA	1865
2089	AACGUGAGCGACUCGCUGG	1619	2089	AACGUGAGCGACUCGCUGG	1619	2111	CCAGCGAGUCGCUCACGUU	1866
2107	GAGAUGCAGUGCUUGGUGG	1620	2107	GAGAUGCAGUGCUUGGUGG	1620	2129	CCACCAAGCACUGCAUCUC	1867
2125	GCCGGAGCGCACGCGCCCA	1621	2125	GCCGGAGCGCACGCGCCCA	1621	2147	UGGGCGCGUGCGCUCCGGC	1868
2143	AGCAUCGUGUGGUACAAAG	1622	2143	AGCAUCGUGUGGUACAAAG	1622	2165	CUUUGUACCACACGAUGCU	1869
2161	GACGAGAGGCUGCUGGAGG	1623	2161	GACGAGAGGCUGCUGGAGG	1623	2183	CCUCCAGCAGCCUCUCGUC	1870
2179	GAAAAGUCUGGAGUCGACU	1624	2179	GAAAAGUCUGGAGUCGACU	1624	2201	AGUCGACUCCAGACUUUUC	1871

2197	UUGGCGGACUCCAACCAGA	1625	2197	UUGGCGGACUCCAACCAGA	1625	2219	UCUGGUUGGAGUCCGCCAA	1872
2215	AAGCUGAGCAUCCAGCGCG	1626	2215	AAGCUGAGCAUCCAGCGCG	1626	2237	CGCGCUGGAUGCUCAGCUU	1873
2233	GUGCGCGAGGAGGAUGCGG	1627	2233	GUGCGCGAGGAGGAUGCGG	1627	2255	CCGCAUCCUCCUCGCGCAC	1874
2251	GGACCGUAUCUGUGCAGCG	1628	2251	GGACCGUAUCUGUGCAGCG	1628	2273	CGCUGCACAGAUACGGUCC	1875
2269	GUGUGCAGACCCAAGGGCU	1629	2269	GUGUGCAGACCCAAGGGCU	1629	2291	AGCCCUUGGGUCUGCACAC	1876
2287	UGCGUCAACUCCUCCGCCA	1630	2287	UGCGUCAACUCCUCCGCCA	1630	2309	UGGCGGAGGAGUUGACGCA	1877
2305	AGCGUGGCCGUGGAAGGCU	1631	2305	AGCGUGGCCGUGGAAGGCU	1631	2327	AGCCUUCCACGGCCACGCU	1878
2323	UCCGAGGAUAAGGGCAGCA	1632	2323	UCCGAGGAUAAGGGCAGCA	1632	2345	UGCUGCCCUUAUCCUCGGA	1879
2341	AUGGAGAUCGUGAUCCUUG	1633	2341	AUGGAGAUCGUGAUCCUUG	1633	2363	CAAGGAUCACGAUCUCCAU	1880
2359	GUCGGUACCGGCGUCAUCG	1634	2359	GUCGGUACCGGCGUCAUCG	1634	2381	CGAUGACGCCGGUACCGAC	1881
2377	GCUGUCUUCUUCUGGGUCC	1635	2377	GCUGUCUUCUUCUGGGUCC	1635	2399	GGACCCAGAAGAAGACAGC	1882
2395	CUCCUCCUCCUCAUCUUCU	1636	2395	CUCCUCCUCCUCAUCUUCU	1636	2417	AGAAGAUGAGGAGGAGGAG	1883
2413	UGUAACAUGAGGAGGCCGG	1637	2413	UGUAACAUGAGGAGGCCGG	1637	2435	CCGGCCUCCUCAUGUUACA	1884
2431	GCCCACGCAGACAUCAAGA	1638	2431	GCCCACGCAGACAUCAAGA	1638	2453	UCUUGAUGUCUGCGUGGGC	1885
2449	ACGGGCUACCUGUCCAUCA	1639	2449	ACGGGCUACCUGUCCAUCA	1639	2471	UGAUGGACAGGUAGCCCGU	1886
2467	AUCAUGGACCCCGGGGAGG	1640	2467	AUCAUGGACCCCGGGGAGG	1640	2489	CCUCCCCGGGGUCCAUGAU	1887
2485	GUGCCUCUGGAGGAGCAAU	1641	2485	GUGCCUCUGGAGGAGCAAU	1641	2507	AUUGCUCCUCCAGAGGCAC	1888
2503	UGCGAAUACCUGUCCUACG	1642	2503	UGCGAAUACCUGUCCUACG	1642	2525	CGUAGGACAGGUAUUCGCA	1889
2521	GAUGCCAGCCAGUGGGAAU	1643	2521	GAUGCCAGCCAGUGGGAAU	1643	2543	AUUCCCACUGGCUGGCAUC	1890
2539	UUCCCCCGAGAGCGGCUGC	1644	2539	UUCCCCCGAGAGCGGCUGC	1644	2561	GCAGCCGCUCUCGGGGGAA	1891
2557	CACCUGGGGAGAGUGCUCG	1645	2557	CACCUGGGGAGAGUGCUCG	1645	2579	CGAGCACUCUCCCCAGGUG	1892
2575	GGCUACGGCGCCUUCGGGA	1646	2575	GGCUACGGCGCCUUCGGGA	1646	2597	UCCCGAAGGCGCCGUAGCC	1893
2593	AAGGUGGUGGAAGCCUCCG	1647	2593	AAGGUGGUGGAAGCCUCCG	1647	2615	CGGAGGCUUCCACCACCUU	1894
2611	GCUUUCGGCAUCCACAAGG	1648	2611	GCUUUCGGCAUCCACAAGG	1648	2633	CCUUGUGGAUGCCGAAAGC	1895
2629	GGCAGCAGCUGUGACACCG	1649	2629	GGCAGCAGCUGUGACACCG	1649	2651	CGGUGUCACAGCUGCUGCC	1896
2647	GUGGCCGUGAAAAUGCUGA	1650	2647	GUGGCCGUGAAAAUGCUGA	1650	2669	UCAGCAUUUUCACGGCCAC	1897
2665	AAAGAGGGCGCCACGGCCA	1651	2665	AAAGAGGGCGCCACGGCCA	1651	2687	UGGCCGUGGCGCCCUCUUU	1898
2683	AGCGAGCAGCGCGCGCUGA	1652	2683	AGCGAGCAGCGCGCGCUGA	1652	2705	UCAGCGCGCGCUGCUCGCU	1899
2701	AUGUCGGAGCUCAAGAUCC	1653	2701	AUGUCGGAGCUCAAGAUCC	1653	2723	GGAUCUUGAGCUCCGACAU	1900
2719	CUCAUUCACAUCGGCAACC	1654	2719	CUCAUUCACAUCGGCAACC	1654	2741	GGUUGCCGAUGUGAAUGAG	1901
2737	CACCUCAACGUGGUCAACC	1655	2737	CACCUCAACGUGGUCAACC	1655	2759	GGUUGACCACGUUGAGGUG	1902
2755	CUCCUCGGGGCGUGCACCA	1656	2755	CUCCUCGGGGCGUGCACCA	1656	2777	UGGUGCACGCCCCGAGGAG	1903
2773	AAGCCGCAGGGCCCCCUCA	1657	2773	AAGCCGCAGGGCCCCCUCA	1657	2795	UGAGGGGGCCCUGCGGCUU	1904

2791	AUGGUGAUCGUGGAGUUCU	1658	2791	AUGGUGAUCGUGGAGUUCU	1658	2813	AGAACUCCACGAUCACCAU	1905
2809	UGCAAGUACGGCAACCUCU	1659	2809	UGCAAGUACGGCAACCUCU	1659	2831	AGAGGUUGCCGUACUUGCA	1906
2827	UCCAACUUCCUGCGCGCCA	1660	2827	UCCAACUUCCUGCGCGCCA	1660	2849	UGGCGCGCAGGAAGUUGGA	1907
2845	AAGCGGGACGCCUUCAGCC	1661	2845	AAGCGGGACGCCUUCAGCC	1661	2867	GGCUGAAGGCGUCCCGCUU	1908
2863	CCCUGCGCGGAGAAGUCUC	1662	2863	CCCUGCGCGGAGAAGUCUC	1662	2885	GAGACUUCUCCGCGCAGGG	1909
2881	CCCGAGCAGCGCGGACGCU	1663	2881	CCCGAGCAGCGCGGACGCU	1663	2903	AGCGUCCGCGCUGCUCGGG	1910
2899	UUCCGCGCCAUGGUGGAGC	1664	2899	UUCCGCGCCAUGGUGGAGC	1664	2921	GCUCCACCAUGGCGCGGAA	1911
2917	CUCGCCAGGCUGGAUCGGA	1665	2917	CUCGCCAGGCUGGAUCGGA	1665	2939	UCCGAUCCAGCCUGGCGAG	1912
2935	AGGCGGCCGGGGAGCAGCG	1666	2935	AGGCGGCCGGGGAGCAGCG	1666	2957	CGCUGCUCCCCGGCCGCCU	1913
2953	GACAGGGUCCUCUUCGCGC	1667	2953	GACAGGGUCCUCUUCGCGC	1667	2975	GCGCGAAGAGGACCCUGUC	1914
2971	CGGUUCUCGAAGACCGAGG	1668	2971	CGGUUCUCGAAGACCGAGG	1668	2993	CCUCGGUCUUCGAGAACCG	1915
2989	GGCGGAGCGAGGCGGGCUU	1669	2989	GGCGGAGCGAGGCGGGCUU	1669	3011	AAGCCCGCCUCGCUCCGCC	1916
3007	UCUCCAGACCAAGAAGCUG	1670	3007	UCUCCAGACCAAGAAGCUG	1670	3029	CAGCUUCUUGGUCUGGAGA	1917
3025	GAGGACCUGUGGCUGAGCC	1671	3025	GAGGACCUGUGGCUGAGCC	1671	3047	GGCUCAGCCACAGGUCCUC	1918
3043	CCGCUGACCAUGGAAGAUC	1672	3043	CCGCUGACCAUGGAAGAUC	1672	3065	GAUCUUCCAUGGUCAGCGG	1919
3061	CUUGUCUGCUACAGCUUCC	1673	3061	CUUGUCUGCUACAGCUUCC	1673	3083	GGAAGCUGUAGCAGACAAG	1920
3079	CAGGUGGCCAGAGGGAUGG	1674	3079	CAGGUGGCCAGAGGGAUGG	1674	3101	CCAUCCCUCUGGCCACCUG	1921
3097	GAGUUCCUGGCUUCCCGAA	1675	3097	GAGUUCCUGGCUUCCCGAA	1675	3119	UUCGGGAAGCCAGGAACUC	1922
3115	AAGUGCAUCCACAGAGACC	1676	3115	AAGUGCAUCCACAGAGACC	1676	3137	GGUCUCUGUGGAUGCACUU	1923
3133	CUGGCUGCUCGGAACAUUC	1677	3133	CUGGCUGCUCGGAACAUUC	1677	3155	GAAUGUUCCGAGCAGCCAG	1924
3151	CUGCUGUCGGAAAGCGACG	1678	3151	CUGCUGUCGGAAAGCGACG	1678	3173	CGUCGCUUUCCGACAGCAG	1925
3169	GUGGUGAAGAUCUGUGACU	1679	3169	GUGGUGAAGAUCUGUGACU	1679	3191	AGUCACAGAUCUUCACCAC	1926
3187	UUUGGCCUUGCCCGGGACA	1680	3187	UUUGGCCUUGCCCGGGACA	1680	3209	UGUCCCGGGCAAGGCCAAA	1927
3205	AUCUACAAAGACCCCGACU	1681	3205	AUCUACAAAGACCCCGACU	1681	3227	AGUCGGGGUCUUUGUAGAU	1928
3223	UACGUCCGCAAGGGCAGUG	1682	3223	UACGUCCGCAAGGGCAGUG	1682	3245	CACUGCCCUUGCGGACGUA	1929
3241	GCCCGGCUGCCCCUGAAGU	1683	3241	GCCCGGCUGCCCCUGAAGU	1683	3263	ACUUCAGGGGCAGCCGGGC	1930
3259	UGGAUGGCCCCUGAAAGCA	1684	3259	UGGAUGGCCCCUGAAAGCA	1684	3281	UGCUUUCAGGGGCCAUCCA	1931
3277	AUCUUCGACAAGGUGUACA	1685	3277	AUCUUCGACAAGGUGUACA	1685	3299	UGUACACCUUGUCGAAGAU	1932
3295	ACCACGCAGAGUGACGUGU	1686	3295	ACCACGCAGAGUGACGUGU	1686	3317	ACACGUCACUCUGCGUGGU	1933
3313	UGGUCCUUUGGGGUGCUUC	1687	3313	UGGUCCUUUGGGGUGCUUC	1687	3335	GAAGCACCCCAAAGGACCA	1934
3331	CUCUGGGAGAUCUUCUCUC	1688	3331	CUCUGGGAGAUCUUCUCUC	1688	3353	GAGAGAAGAUCUCCCAGAG	1935
3349	CUGGGGGCCUCCCCGUACC	1689	3349	CUGGGGGCCUCCCCGUACC	1689	3371	GGUACGGGGAGGCCCCCAG	1936
3367	CCUGGGGUGCAGAUCAAUG	1690	3367	CCUGGGGUGCAGAUCAAUG	1690	3389	CAUUGAUCUGCACCCCAGG	1937

3385	GAGGAGUUCUGCCAGCGCG	1691	3385	GAGGAGUUCUGCCAGCGCG	1691	3407	CGCGCUGGCAGAACUCCUC	1938
3403	GUGAGAGACGGCACAAGGA	1692	3403	GUGAGAGACGGCACAAGGA	1692	3425	UCCUUGUGCCGUCUCUCAC	1939
3421	AUGAGGGCCCCGGAGCUGG	1693	3421	AUGAGGGCCCCGGAGCUGG	1693	3443	CCAGCUCCGGGGCCCUCAU	1940
3439	GCCACUCCCGCCAUACGCC	1694	3439	GCCACUCCCGCCAUACGCC	1694	3461	GGCGUAUGGCGGGAGUGGC	1941
3457	CACAUCAUGCUGAACUGCU	1695	3457	CACAUCAUGCUGAACUGCU	1695	3479	AGCAGUUCAGCAUGAUGUG	1942
3475	UGGUCCGGAGACCCCAAGG	1696	3475	UGGUCCGGAGACCCCAAGG	1696	3497	CCUUGGGGUCUCCGGACCA	1943
3493	GCGAGACCUGCAUUCUCGG	1697	3493	GCGAGACCUGCAUUCUCGG	1697	3515	CCGAGAAUGCAGGUCUCGC	1944
3511	GACCUGGUGGAGAUCCUGG	1698	3511	GACCUGGUGGAGAUCCUGG	1698	3533	CCAGGAUCUCCACCAGGUC	1945
3529	GGGGACCUGCUCCAGGGCA	1699	3529	GGGGACCUGCUCCAGGGCA	1699	3551	UGCCCUGGAGCAGGUCCCC	1946
3547	AGGGGCCUGCAAGAGGAAG	1700	3547	AGGGGCCUGCAAGAGGAAG	1700	3569	CUUCCUCUUGCAGGCCCCU	1947
3565	GAGGAGGUCUGCAUGGCCC	1701	3565	GAGGAGGUCUGCAUGGCCC	1701	3587	GGGCCAUGCAGACCUCCUC	1948
3583	CCGCGCAGCUCUCAGAGCU	1702	3583	CCGCGCAGCUCUCAGAGCU	1702	3605	AGCUCUGAGAGCUGCGCGG	1949
3601	UCAGAAGAGGGCAGCUUCU	1703	3601	UCAGAAGAGGGCAGCUUCU	1703	3623	AGAAGCUGCCCUCUUCUGA	1950
3619	UCGCAGGUGUCCACCAUGG	1704	3619	UCGCAGGUGUCCACCAUGG	1704	3641	CCAUGGUGGACACCUGCGA	1951
3637	GCCCUACACAUCGCCCAGG	1705	3637	GCCCUACACAUCGCCCAGG	1705	3659	CCUGGGCGAUGUGUAGGGC	1952
3655	GCUGACGCUGAGGACAGCC	1706	3655	GCUGACGCUGAGGACAGCC	1706	3677	GGCUGUCCUCAGCGUCAGC	1953
3673	CCGCCAAGCCUGCAGCGCC	1707	3673	CCGCCAAGCCUGCAGCGCC	1707	3695	GGCGCUGCAGGCUUGGCGG	1954
3691	CACAGCCUGGCCGCCAGGU	1708	3691	CACAGCCUGGCCGCCAGGU	1708	3713	ACCUGGCGGCCAGGCUGUG	1955
3709	UAUUACAACUGGGUGUCCU	1709	3709	UAUUACAACUGGGUGUCCU	1709	3731	AGGACACCCAGUUGUAAUA	1956
3727	UUUCCCGGGUGCCUGGCCA	1710	3727	UUUCCCGGGUGCCUGGCCA	1710	3749	UGGCCAGGCACCCGGGAAA	1957
3745	AGAGGGGCUGAGACCCGUG	1711	3745	AGAGGGGCUGAGACCCGUG	1711	3767	CACGGGUCUCAGCCCCUCU	1958
3763	GGUUCCUCCAGGAUGAAGA	1712	3763	GGUUCCUCCAGGAUGAAGA	1712	3785	UCUUCAUCCUGGAGGAACC	1959
3781	ACAUUUGAGGAAUUCCCCA	1713	3781	ACAUUUGAGGAAUUCCCCA	1713	3803	UGGGGAAUUCCUCAAAUGU	1960
3799	AUGACCCCAACGACCUACA	1714	3799	AUGACCCCAACGACCUACA	1714	3821	UGUAGGUCGUUGGGGUCAU	1961
3817	AAAGGCUCUGUGGACAACC	1715	3817	AAAGGCUCUGUGGACAACC	1715	3839	GGUUGUCCACAGAGCCUUU	1962
3835	CAGACAGACAGUGGGAUGG	1716	3835	CAGACAGACAGUGGGAUGG	1716	3857	CCAUCCCACUGUCUGUCUG	1963
3853	GUGCUGGCCUCGGAGGAGU	1717	3853	GUGCUGGCCUCGGAGGAGU	1717	3875	ACUCCUCCGAGGCCAGCAC	1964
3871	UUUGAGCAGAUAGAGAGCA	1718	3871	UUUGAGCAGAUAGAGAGCA	1718	3893	UGCUCUCUAUCUGCUCAAA	1965
3889	AGGCAUAGACAAGAAAGCG	1719	3889	AGGCAUAGACAAGAAAGCG	1719	3911	CGCUUUCUUGUCUAUGCCU	1966
3907	GGCUUCAGGUAGCUGAAGC	1720	3907	GGCUUCAGGUAGCUGAAGC	1720	3929	GCUUCAGCUACCUGAAGCC	1967
3925	CAGAGAGAGAGAAGGCAGC	1721	3925	CAGAGAGAGAGAAGGCAGC	1721	3947	GCUGCCUUCUCUCUCUCUG	1968
3943	CAUACGUCAGCAUUUUCUU	1722	3943	CAUACGUCAGCAUUUUCUU	1722	3965	AAGAAAAUGCUGACGUAUG	1969
3961	UCUCUGCACUUAUAAGAAA	1723	3961	UCUCUGCACUUAUAAGAAA	1723	3983	UUUCUUAUAAGUGCAGAGA	1970

3979	AGAUCAAAGACUUUAAGAC	1724	3979	AGAUCAAAGACUUUAAGAC	1724	4001	GUCUUAAAGUCUUUGAUCU	1971
3997	CUUUCGCUAUUUCUUCUAC	1725	3997	CUUUCGCUAUUUCUUCUAC	1725	4019	GUAGAAGAAAUAGCGAAAG	1972
4015	CUGCUAUCUACUACAAACU	1726	4015	CUGCUAUCUACUACAAACU	1726	4037	AGUUUGUAGUAGAUAGCAG	1973
4033	UUCAAAGAGGAACCAGGAG	1727	4033	UUCAAAGAGGAACCAGGAG	1727	4055	CUCCUGGUUCCUCUUUGAA	1974
4051	GGACAAGAGGAGCAUGAAA	1728	4051	GGACAAGAGGAGCAUGAAA	1728	4073	UUUCAUGCUCCUCUUGUCC	1975
4069	AGUGGACAAGGAGUGUGAC	1729	4069	AGUGGACAAGGAGUGUGAC	1729	4091	GUCACACUCCUUGUCCACU	1976
4087	CCACUGAAGCACCACAGGG	1730	4087	CCACUGAAGCACCACAGGG	1730	4109	CCCUGUGGUGCUUCAGUGG	1977
4105	GAGGGGUUAGGCCUCCGGA	1731	4105	GAGGGGUUAGGCCUCCGGA	1731	4127	UCCGGAGGCCUAACCCCUC	1978
4123	AUGACUGCGGGCAGGCCUG	1732	4123	AUGACUGCGGGCAGGCCUG	1732	4145	CAGGCCUGCCCGCAGUCAU	1979
4141	GGAUAAUAUCCAGCCUCCC	1733	4141	GGAUAAUAUCCAGCCUCCC	1733	4163	GGGAGGCUGGAUAUUAUCC	1980
4159	CACAAGAAGCUGGUGGAGC	1734	4159	CACAAGAAGCUGGUGGAGC	1734	4181	GCUCCACCAGCUUCUUGUG	1981
4177	CAGAGUGUUCCCUGACUCC	1735	4177	CAGAGUGUUCCCUGACUCC	1735	4199	GGAGUCAGGGAACACUCUG	1982
4195	CUCCAAGGAAAGGGAGACG	1736	4195	CUCCAAGGAAAGGGAGACG	1736	4217	CGUCUCCCUUUCCUUGGAG	1983
4213	GCCCUUUCAUGGUCUGCUG	1737	4213	GCCCUUUCAUGGUCUGCUG	1737	4235	CAGCAGACCAUGAAAGGGC	1984
4231	GAGUAACAGGUGCCUUCCC	1738	4231	GAGUAACAGGUGCCUUCCC	1738	4253	GGGAAGGCACCUGUUACUC	1985
4249	CAGACACUGGCGUUACUGC	1739	4249	CAGACACUGGCGUUACUGC	1739	4271	GCAGUAACGCCAGUGUCUG	1986
4267	CUUGACCAAAGAGCCCUCA	1740	4267	CUUGACCAAAGAGCCCUCA	1740	4289	UGAGGGCUCUUUGGUCAAG	1987
4285	AAGCGGCCCUUAUGCCAGC	1741	4285	AAGCGGCCCUUAUGCCAGC	1741	4307	GCUGGCAUAAGGGCCGCUU	1988
4303	CGUGACAGAGGGCUCACCU	1742	4303	CGUGACAGAGGGCUCACCU	1742	4325	AGGUGAGCCCUCUGUCACG	1989
4321	UCUUGCCUUCUAGGUCACU	1743	4321	UCUUGCCUUCUAGGUCACU	1743	4343	AGUGACCUAGAAGGCAAGA	1990
4339	UUCUCACAAUGUCCCUUCA	1744	4339	UUCUCACAAUGUCCCUUCA	1744	4361	UGAAGGGACAUUGUGAGAA	1991
4357	AGCACCUGACCCUGUGCCC	1745	4357	AGCACCUGACCCUGUGCCC	1745	4379	GGGCACAGGGUCAGGUGCU	1992
4375	CGCCGAUUAUUCCUUGGUA	1746	4375	CGCCGAUUAUUCCUUGGUA	1746	4397	UACCAAGGAAUAAUCGGCG	1993
4393	AAUAUGAGUAAUACAUCAA	1747	4393	AAUAUGAGUAAUACAUCAA	1747	4415	UUGAUGUAUUACUCAUAUU	1994
4411	AAGAGUAGUAUUAAAAGCU	1748	4411	AAGAGUAGUAUUAAAAGCU	1748	4433	AGCUUUUAAUACUACUCUU	1995
4429	UAAUUAAUCAUGUUUAUAA	1749	4429	UAAUUAAUCAUGUUUAUAA	1749	4451	UUAUAAACAUGAUUAAUUA	1996

The 3'-ends of the Upper sequence and the Lower sequence of the siNA construct can include an overhang sequence, for example about 1, 2, 3, or 4 nucleotides in length, preferably 2 nucleotides in length, wherein the overhanging sequence of the lower sequence is optionally complementary to a portion of the target sequence. The upper sequence is also referred to as the sense strand, whereas the lower sequence is also referred to as the antisense strand. The upper and lower sequences in the Table can further comprise a chemical modification having Formulae I-VII or any combination thereof.

Table III: VEGF and VEGFr Synthetic Modified siNA constructs

VEGFR1

Target Pos	Target	Seq ID	Aliases	Sequence	Seq ID
296	GCUGUCUGCUUCUCACAGGAUCU	1997	FLT1:298U21 siRNA sense	UGUCUGCUUCUCACAGGAUTT	2020
1954	GAAGGAGAGGACCUGAAACUGUC	1998	FLT1:1956U21 siRNA sense	AGGAGAGGACCUGAAACUGTT	2021
1955	AAGGAGAGGACCUGAAACUGUCU	1999	FLT1:1957U21 siRNA sense	GGAGAGGACCUGAAACUGUTT	2022
2785	GCAUUUGGCAUUAAGAAAUCACC	2000	FLT1:2787U21 siRNA sense	AUUUGGCAUUAAGAAAUCATT	2023
296	GCUGUCUGCUUCUCACAGGAUCU	1997	FLT1:316L21 siRNA (298C) antisense	AUCCUGUGAGAAGCAGACATT	2024
1954	GAAGGAGAGGACCUGAAACUGUC	1998	FLT1:1974L21 siRNA (1956C) antisense	CAGUUUCAGGUCCUCUCCUTT	2025
1955	AAGGAGAGGACCUGAAACUGUCU	1999	FLT1:1975L21 siRNA (1957C) antisense	ACAGUUUCAGGUCCUCUCCTT	2026
2785	GCAUUUGGCAUUAAGAAAUCACC	2000	FLT1:2805L21 siRNA (2787C) antisense	UGAUUUCUUAAUGCCAAAUTT	2027
296	GCUGUCUGCUUCUCACAGGAUCU	1997	FLT1:298U21 siRNA stab04 sense	B uGucuGcuucucAcAGGAuTT B	2028
1954	GAAGGAGAGGACCUGAAACUGUC	1998	FLT1:1956U21 siRNA stab04 sense	B AGGAGAGGAccuGAAAcuGTT B	2029
1955	AAGGAGAGGACCUGAAACUGUCU	1999	FLT1:1957U21 siRNA stab04 sense	B GGAGAGGAccuGAAAcuGuTT B	2030
2785	GCAUUUGGCAUUAAGAAAUCACC	2000	FLT1:2787U21 siRNA stab04 sense	B AuuuGGcAuuAAGAAAucATT B	2031
296	GCUGUCUGCUUCUCACAGGAUCU	1997	FLT1:316L21 siRNA (298C) stab05 antisense	AuccuGuGAGAAGcAGAcATsT	2032
1954	GAAGGAGAGGACCUGAAACUGUC	1998	FLT1:1974L21 siRNA (1956C) stab05 antisense	cAGuuucAGGuccucuccuTsT	2033
1955	AAGGAGAGGACCUGAAACUGUCU	1999	FLT1:1975L21 siRNA (1957C) stab05 antisense	AcAGuuucAGGuccucuccTsT	2034
2785	GCAUUUGGCAUUAAGAAAUCACC	2000	FLT1:2805L21 siRNA (2787C) stab05 antisense	uGAuuucuuAAuGccAAAuTsT	2035
296	GCUGUCUGCUUCUCACAGGAUCU	1997	FLT1:298U21 siRNA stab07 sense	B uGucuGcuucucAcAGGAuTT B	2036
1954	GAAGGAGAGGACCUGAAACUGUC	1998	FLT1:1956U21 siRNA stab07 sense	B AGGAGAGGAccuGAAAcuGTT B	2037
1955	AAGGAGAGGACCUGAAACUGUCU	1999	FLT1:1957U21 siRNA stab07 sense	B GGAGAGGAccuGAAAcuGuTT B	2038
2785	GCAUUUGGCAUUAAGAAAUCACC	2000	FLT1:2787U21 siRNA stab07 sense	B AuuuGGcAuuAAGAAAucATT B	2039
296	GCUGUCUGCUUCUCACAGGAUCU	1997	FLT1:316L21 siRNA (298C) stab11 antisense	AuccuGuGAGAAGcAGAcATsT	2040
1954	GAAGGAGAGGACCUGAAACUGUC	1998	FLT1:1974L21 siRNA (1956C) stab11 antisense	cAGuuucAGGuccucuccuTsT	2041
1955	AAGGAGAGGACCUGAAACUGUCU	1999	FLT1:1975L21 siRNA (1957C) stab11 antisense	AcAGuuucAGGuccucuccTsT	2042
2785	GCAUUUGGCAUUAAGAAAUCACC	2000	FLT1:2805L21 siRNA (2787C) stab11 antisense	uGAuuucuuAAuGccAAAuTsT	2043

VEGFR1

Target	SeqID	RPI#	Alias	Sequence	SeqID
AACUGAGUUUAAAAGGCACCCAG	2009	29694	FLT1:349U21 siRNA stab01 sense	CsUsGsAsGsUUUAAAAGGCACCCTsT	2092
AACAACCACAAAAUACAACAAGA	2010	29695	FLT1:2340U21 siRNA stab01 sense	CsAsAsCsCsACAAAAUACAACAATsT	2093
AGCCUGGAAAGAAUCAAAACCUU	2011	29696	FLT1:3912U21 siRNA stab01 sense	CsCsUsGsGsAAAGAAUCAAAACCTsT	2094
AAGCAAGGAGGGCCUCUGAUGGU	2012	29697	FLT1:2949U21 siRNA stab01 sense	GsCsAsAsGsGAGGGCCUCUGAUGTsT	2095
AACUGAGUUUAAAAGGCACCCAG	2009	29698	FLT1:369L21 siRNA (349C) stab01 sense	GsGsGsUsGsCCUUUUAAACUCAGTsT	2096
AACAACCACAAAAUACAACAAGA	2010	29699	FLT1:2358L21 siRNA (2340C) stab01 sense	UsUsGsUsUsGUAUUUUGUGGUUGTsT	2097
AGCCUGGAAAGAAUCAAAACCUU	2011	29700	FLT1:3932L21 siRNA (3912C) stab01 sense	GsGsUsUsUsUGAUUCUUUCCAGGTsT	2098
AAGCAAGGAGGGCCUCUGAUGGU	2012	29701	FLT1:2969L21 siRNA (2949C) stab01 sense	CsAsUsCsAsGAGGCCCUCCUUGCTsT	2099
AACUGAGUUUAAAAGGCACCCAG	2009	29702	FLT1:349U21 siRNA stab03 sense	csusGsAsGuuuAAAAGGcAcscscsTsT	2100
AACAACCACAAAAUACAACAAGA	2010	29703	FLT1:2340U21 siRNA stab03 sense	csAsAscscAcAAAAuAcAAcsAsAsTsT	2101
AGCCUGGAAAGAAUCAAAACCUU	2011	29704	FLT1:3912U21 siRNA stab03 sense	cscsusGsGsAAAGAAucAAAAscscsTsT	2102
AAGCAAGGAGGGCCUCUGAUGGU	2012	29705	FLT1:2949U21 siRNA stab03 sense	GscsAsAsGGAGGGccucuGAsusGsTsT	2103
AACUGAGUUUAAAAGGCACCCAG	2009	29706	FLT1:369L21 siRNA (349C) stab02 antisense	GsGsGsUsGsCsCsUsUsUsUsAsAsAsCsUsCsAsGsTsT	2104
AACAACCACAAAAUACAACAAGA	2010	29707	FLT1:2358L21 siRNA (2340C) stab02 antisense	UsUsGsUsUsGsUsAsUsUsUsUsGsUsGsGsUsUsGsTsT	2105
AGCCUGGAAAGAAUCAAAACCUU	2011	29708	FLT1:3932L21 siRNA (3912C) stab02 antisense	GsGsUsUsUsUsGsAsUsUsCsUsUsUsCsCsAsGsGsTsT	2106
AAGCAAGGAGGGCCUCUGAUGGU	2012	29709	FLT1:2969L21 siRNA (2949C) stab02 antisense	CsAsUsCsAsGsAsGsGsCsCsCsUsCsCsUsUsGsCsTsT	2107
AACAACCACAAAAUACAACAAGA	2010	29981	FLT1:2340U21 siRNA Native sense	CAACCACAAAAUACAACAAGA	2108
AACAACCACAAAAUACAACAAGA	2010	29982	FLT1:2358L21 siRNA (2340C) Native antisense	UUGUUGUAUUUUGUGGUUGUU	2109
AACAACCACAAAAUACAACAAGA	2010	29983	FLT1:2342U21 siRNA stab01 inv	AsAsCsAsAsCAUAAAACACCAACTsT	2110
AACAACCACAAAAUACAACAAGA	2010	29984	FLT1:2358L21 siRNA (2340C) stab01 inv	GsUsUsGsGsUGUUUUAUGUUGUUTsT	2111
AACAACCACAAAAUACAACAAGA	2010	29985	FLT1:2342U21 siRNA stab03 inv	AsAscsAsAcAuAAAAcAccAsAscsTsT	2112
AACAACCACAAAAUACAACAAGA	2010	29986	FLT1:2358L21 siRNA (2340C) stab02 inv	GsUsUsGsGsUsGsUsUsUsUsAsUsGsUsUsGsUsUsTsT	2113

AACAACCACAAAAUACAACAAGA	2010	29987	FLT1:2340U21 siRNA inv Native sense	AGAACAACAUAAAACACCAAC	2114
AACAACCACAAAAUACAACAAGA	2010	29988	FLT1:2358L21 siRNA (2340C) inv Native	UUGUUGGUGUUUUAUGUUGUU	2115
AACAACCACAAAAUACAACAAGA	2010	30075	FLT1:2340U21 siRNA sense	CAACCACAAAAUACAACAATT	2116
AACAACCACAAAAUACAACAAGA	2010	30076	FLT1:2358L21 siRNA (2340C) antisense	UUGUUGUAUUUUGUGGUUGTT	2117
AACAACCACAAAAUACAACAAGA	2010	30077	FLT1:2342U21 siRNA inv	AGAACAACAUAAAACACCATT	2118
AACAACCACAAAAUACAACAAGA	2010	30078	FLT1:2358L21 siRNA (2340C) inv	UUGUUGGUGUUUUAUGUUGTT	2119
AACAACCACAAAAUACAACAAGA	2010	30187	FLT1:2358L21 siRNA (2340C) 2'-F U,C antisense	uuGuuGuAuuuuGuGGuuGTT	2120
AACAACCACAAAAUACAACAAGA	2010	30190	FLT1:2358L21 siRNA (2340C) X = nitroindole antisense	uuGuuGuAuuuuGuGGuuGXX	2121
AACAACCACAAAAUACAACAAGA	2010	30193	FLT1:2358L21 siRNA (2340C) Z = nitropyrole antisense	uuGuuGuAuuuuGuGGuuGZZ	2122
AACAACCACAAAAUACAACAAGA	2010	30196	FLT1:2340U21 siRNA sense iB caps w/2'FY's sense	B cAAccAcAAAAuAcAAcAATT B	2123
AACAACCACAAAAUACAACAAGA	2010	30199	FLT1:2340U21 siRNA sense iB caps sense	cAAccAcAAAAuAcAAcAATT	2124
AACAACCACAAAAUACAACAAGA	2010	30340	FLT1:2358L21 siRNA (2340C) X = 3'dT antisense	uuGuuGuAuuuuGuGGuuGTX	2125
AACAACCACAAAAUACAACAAGA	2010	30341	FLT1:2358L21 siRNA (2340C) X = glyceryl antisense	uuGuuGuAuuuuGuGGuuGTX	2126
AACAACCACAAAAUACAACAAGA	2010	30342	FLT1:2358L21 siRNA (2340C) U = 3'OMeU antisense	uuGuuGuAuuuuGuGGuuGTU	2127
AACAACCACAAAAUACAACAAGA	2010	30343	FLT1:2358L21 siRNA (2340C) t = L- dT antisense	uuGuuGuAuuuuGuGGuuGTt	2128
AACAACCACAAAAUACAACAAGA	2010	30344	FLT1:2358L21 siRNA (2340C) u = L-rU antisense	uuGuuGuAuuuuGuGGuuGTu	2129
AACAACCACAAAAUACAACAAGA	2010	30345	FLT1:2358L21 siRNA (2340C) D = idT antisense	uuGuuGuAuuuuGuGGuuGTD	2130
AACAACCACAAAAUACAACAAGA	2010	30346	FLT1:2358L21 siRNA (2340C) X = 3'dT antisense	uuGuuGuAuuuuGuGGuuGXT	2131
AACAACCACAAAAUACAACAAGA	2010	30416	FLT1:2358L21 siRNA (2340C) TsT antisense	uuGuuGuAuuuuGuGGuuGTsT	2132
UCGUGUAAGGAGUGGACCAUCAU	2013	30777	FLT1:1184U21 siRNA stab04 sense	B GuGuAAGGAGuGGAccAucTT B	2133
UUACGGAGUAUUGCUGUGGGAAA	2014	30778	FLT1:3503U21 siRNA stab04 sense	B AcGGAGuAuuGcuGuGGGATT B	2134
UAGCAGGCCUAAGACAUGUGAGG	2015	30779	FLT1:4715U21 siRNA stab04 sense	B GcAGGccuAAGAcAuGuGATT B	2135
AGCAAAAAGCAAGGGAGAAAAGA	2016	30780	FLT1:4753U21 siRNA stab04 sense	B cAAAAAGcAAGGGAGAAAATT B	2136

UCGUGUAAGGAGUGGACCAUCAU	2013	30781	FLT1:1202L21 siRNA (1184C) stab05 antisense	GAuGGuccAcuccuuAcAcTsT	2137
UUACGGAGUAUUGCUGUGGGAAA	2014	30782	FLT1:3521L21 siRNA (3503C) stab05 antisense	ucccAcAGcAAuAcuccGuTsT	2138
UAGCAGGCCUAAGACAUGUGAGG	2015	30783	FLT1:4733L21 siRNA (4715C) stab05 antisense	ucAcAuGucuuAGGccuGcTsT	2139
AGCAAAAAGCAAGGGAGAAAAGA	2016	30784	FLT1:4771L21 siRNA (4753C) stab05 antisense	uuuucucccuuGcuuuuuGTsT	2140
AACAACCACAAAAUACAACAAGA	2010	30955	FLT1:2340U21 siRNA stab07 sense	B c*AA*cc*A*c*AAAA*u*A*c*AA*c*AA*TT B	2141
AACAACCACAAAAUACAACAAGA	2010	30956	FLT1:2358L21 siRNA (2340C) stab08 antisense	uuGuuGuAuuuuGuGGuuGTsT	2142
AACAACCACAAAAUACAACAAGA	2010	30963	FLT1:2340U21 siRNA inv	AACAACAUAAAACACCAACTT	2143
AACAACCACAAAAUACAACAAGA	2010	30964	FLT1:2358L21 siRNA (2340C) inv	GUUGGUGUUUUAUGUUGUUTT	2144
AACAACCACAAAAUACAACAAGA	2010	30965	FLT1:2340U21 siRNA stab04 inv	B AAcAAcAuAAAAcAccAAcTT B	2145
AACAACCACAAAAUACAACAAGA	2010	30966	FLT1:2358L21 siRNA (2340C) stab05 inv	GuuGGuGuuuuAuGuuGuuTsT	2146
AACAACCACAAAAUACAACAAGA	2010	30967	FLT1:2340U21 siRNA stab07 inv	B *AA*c*AA*c*A*u*AAAA*c*A*cc*AA*cTT B	2147
AACAACCACAAAAUACAACAAGA	2010	30968	FLT1:2358L21 siRNA (2340C) stab08 inv	GuuGGuGuuuuAuGuuGuuTsT	2148
AACUGAGUUUAAAAGGCACCCAG	2009	31182	FLT1:349U21 siRNA TT sense	CUGAGUUUAAAAGGCACCCTT	2149
AAGCAAGGAGGGCCUCUGAUGGU	2012	31183	FLT1:2949U21 siRNA TT antisense	GCAAGGAGGGCCUCUGAUGTT	2150
AGCCUGGAAAGAAUCAAAACCUU	2011	31184	FLT1:3912U21 siRNA TT sense	CCUGGAAAGAAUCAAAACCTT	2151
AACUGAGUUUAAAAGGCACCCAG	2009	31185	FLT1:367L21 siRNA (349C) TT antisense	GGGUGCCUUUUAAACUCAGTT	2152
AAGCAAGGAGGGCCUCUGAUGGU	2012	31186	FLT1:2967L21 siRNA (2949C) TT sense	CAUCAGAGGCCCUCCUUGCTT	2153
AGCCUGGAAAGAAUCAAAACCUU	2011	31187	FLT1:3930L21 siRNA (3912C) TT antisense	GGUUUUGAUUCUUUCCAGGTT	2154
AACUGAGUUUAAAAGGCACCCAG	2009	31188	FLT1:349U21 siRNA stab04 sense	B cuGAGuuuAAAAGGcAcccTT B	2155
AAGCAAGGAGGGCCUCUGAUGGU	2012	31189	FLT1:2949U21 siRNA stab04 sense	B GcAAGGAGGGccucuGAuGTT B	2156
AGCCUGGAAAGAAUCAAAACCUU	2011	31190	FLT1:3912U21 siRNA stab04 sense	B ccuGGAAAGAAucAAAAccTT B	2157
AACUGAGUUUAAAAGGCACCCAG	2009	31191	FLT1:367L21 siRNA (349C) stab05 antisense	GGGuGccuuuuAAAcucAGTsT	2158
AAGCAAGGAGGGCCUCUGAUGGU	2012	31192	FLT1:2967L21 siRNA (2949C) stab05 antisense	cAucAGAGGcccuccuuGcTsT	2159
AGCCUGGAAAGAAUCAAAACCUU	2011	31193	FLT1:3930L21 siRNA (3912C) stab05 antisense	GGuuuuGAuucuuuccAGGTsT	2160

AACUGAGUUUAAAAGGCACCCAG	2009	31194	FLT1:349U21 siRNA stab07 sense	B cuGAGuuuAAAAGGcAcccTT B	2161
AAGCAAGGAGGGCCUCUGAUGGU	2012	31195	FLT1:2949U21 siRNA stab07 sense	B GcAAGGAGGGccucuGAuGTT B	2162
AGCCUGGAAAGAAUCAAAACCUU	2011	31196	FLT1:3912U21 siRNA stab07 sense	B ccuGGAAAGAAucAAAAccTT B	2163
AACUGAGUUUAAAAGGCACCCAG	2009	31197	FLT1:367L21 siRNA (349C) stab08 antisense	GGGuGccuuuuAAAcucAGTsT	2164
AAGCAAGGAGGGCCUCUGAUGGU	2012	31198	FLT1:2967L21 siRNA (2949C) stab08 antisense	cAucAGAGGcccuccuuGcTsT	2165
AGCCUGGAAAGAAUCAAAACCUU	2011	31199	FLT1:3930L21 siRNA (3912C) stab08 antisense	GGuuuuGAuucuuuccAGGTsT	2166
AACUGAGUUUAAAAGGCACCCAG	2009	31200	FLT1:349U21 siRNA inv TT	CCCACGGAAAAUUUGAGUCTT	2167
AAGCAAGGAGGGCCUCUGAUGGU	2012	31201	FLT1:2949U21 siRNA inv TT	GUAGUCUCCGGGAGGAACGTT	2168
AGCCUGGAAAGAAUCAAAACCUU	2011	31202	FLT1:3912U21 siRNA inv TT	CCAAAACUAAGAAAGGUCCTT	2169
AACUGAGUUUAAAAGGCACCCAG	2009	31203	FLT1:367L21 siRNA (349C) inv TT	GACUCAAAUUUUCCGUGGGTT	2170
AAGCAAGGAGGGCCUCUGAUGGU	2012	31204	FLT1:2967L21 siRNA (2949C) inv TT	CGUUCCUCCCGGAGACUACTT	2171
AGCCUGGAAAGAAUCAAAACCUU	2011	31205	FLT1:3930L21 siRNA (3912C) inv TT	GGACCUUUCUUAGUUUUGGTT	2172
AACUGAGUUUAAAAGGCACCCAG	2009	31206	FLT1:349U21 siRNA stab04 inv	B cccAcGGAAAAuuuGAGucTT B	2173
AAGCAAGGAGGGCCUGUGAUGGU	2012	31207	FLT1:2949U21 siRNA stab04 inv	B GuAGucuccGGGAGGAAcGTT B	2174
AGCCUGGAAAGAAUCAAAACCUU	2011	31208	FLT1:3912U21 siRNA stab04 inv	B ccAAAAcuAAGAAAGGuccTT B	2175
AACUGAGUUUAAAAGGCACCCAG	2009	31209	FLT1:367L21 siRNA (349C) stab05 inv	GAcucAAAuuuuccGuGGGTsT	2176
AAGCAAGGAGGGCCUCUGAUGGU	2012	31210	FLT1:2967L21 siRNA (2949C) stab05 inv	cGuuccucccGGAGAcuAcTsT	2177
AGCCUGGAAAGAAUCAAAACCUU	2011	31211	FLT1:3930L21 siRNA (3912C) stab05 inv	GGAccuuucuuAGuuuuGGTsT	2178
AACUGAGUUUAAAAGGCACCCAG	2009	31212	FLT1:349U21 siRNA stab07 inv	B cccAcGGAAAAuuuGAGucTT B	2179
AAGCAAGGAGGGCCUCUGAUGGU	2012	31213	FLT1:2949U21 siRNA stab07 inv	B GuAGucuccGGGAGGAAcGTT B	2180
AGCCUGGAAAGAAUCAAAACCUU	2011	31214	FLT1:3912U21 siRNA stab07 inv	B ccAAAAcuAAGAAAGGuccTT B	2181
AACUGAGUUUAAAAGGCACCCAG	2009	31215	FLT1:367L21 siRNA (349C) stab08 inv	GAcucAAAuuuuccGuGGGTsT	2182
AAGCAAGGAGGGCCUCUGAUGGU	2012	31216	FLT1:2967L21 siRNA (2949C) stab08 inv	cGuuccucccGGAGAcuAcTsT	2183
AGCCUGGAAAGAAUCAAAACCUU	2011	31217	FLT1:3930L21 siRNA (3912C) stab08 inv	GGAccuuucuuAGuuuuGGTsT	2184
AACUGAGUUUAAAAGGCACCCAG	2009	31270	FLT1:349U21 siRNA stab09 sense	B CUGAGUUUAAAAGGCACCCTT B	2185
AAGCAAGGAGGGCCUCUGAUGGU	2012	31271	FLT1:2949U21 siRNA stab09	B GCAAGGAGGGCCUCUGAUGTT B	2186

			sense		
AGCCUGGAAAGAAUCAAAACCUU	2011	31272	FLT1:3912U21 siRNA stab09 sense	B CCUGGAAAGAAUCAAAACCTT B	2187
AACUGAGUUUAAAAGGCACCCAG	2009	31273	FLT1:367L21 siRNA (349C) stab10 antisense	GGGUGCCUUUUAAACUCAGTsT	2188
AAGCAAGGAGGGCCUCUGAUGGU	2012	31274	FLT1:2967L21 siRNA (2949C) stab10 antisense	CAUCAGAGGCCCUCCUUGCTsT	2189
AGCCUGGAAAGAAUCAAAACCUU	2011	31275	FLT1:3930L21 siRNA (3912C) stab10 antisense	GGUUUUGAUUCUUUCCAGGTsT	2190
AACUGAGUUUAAAAGGCACCCAG	2009	31276	FLT1:349U21 siRNA stab09 inv	B CCCACGGAAAAUUUGAGUCTT B	2191
AAGCAAGGAGGGCCUCUGAUGGU	2012	31277	FLT1:2949U21 siRNA stab09 inv	B GUAGUCUCCGGGAGGAACGTT B	2192
AGCCUGGAAAGAAUCAAAACCUU	2011	31278	FLT1:3912U21 siRNA stab09 inv	B CCAAAACUAAGAAAGGUCCTT B	2193
AACUGAGUUUAAAAGGCACCCAG	2009	31279	FLT1:367L21 siRNA (349C) stab10 inv	GACUCAAAUUUUCCGUGGGTsT	2194
AAGCAAGGAGGGCCUCUGAUGGU	2012	31280	FLT1:2967L21 siRNA (2949C) stab10 inv	CGUUCCUCCCGGAGACUACTsT	2195
AGCCUGGAAAGAAUCAAAACCUU	2011	31281	FLT1:3930L21 siRNA (3912C) stab10 inv	GGACCUUUCUUAGUUUUGGTsT	2196
AACAACCACAAAAUACAACAAGA	2010	31424	FLT1:2358L21 siRNA (2340C) stab11 X = 3'-BrdU antisense	uuGuuGuAuuuuGuGGuuGXsX	2197
AAGCAAGGAGGGCCUCUGAUGGU	2012	31425	FLT1:2967L21 siRNA (2949C) stab11 X = 3'-BrdU sense	cAucAGAGGcccuccuuGcXsX	2198
AACAACCACAAAAUACAACAAGA	2010	31442	FLT1:2358L21 siRNA (2340C) stab11 X = 3'-BrdU antisense	uuGuuGuAuuuuGuGGuuGXsT	2199
AAGCAAGGAGGGCCUCUGAUGGU	2012	31443	FLT1:2967L21 siRNA (2949C) stab11 X = 3'-BrdU sense	cAucAGAGGcccuccuuGcXsT	2200
AACAACCACAAAAUACAACAAGA	2010	31449	FLT1:2340U21 siRNA stab09 sense	B CAACCACAAAAUACAACAATT B	2201
AACAACCACAAAAUACAACAAGA	2010	31450	FLT1:2340U21 siRNA inv stab09 sense	B AACAACAUAAAACACCAACTT B	2202
AACAACCACAAAAUACAACAAGA	2010	31451	FLT1:2358L21 siRNA (2340C) stab10 antisense	UUGUUGUAUUUUGUGGUUGTsT	2203
AACAACCACAAAAUACAACAAGA	2010	31452	FLT1:2358L21 siRNA (2340C) inv stab10 antisense	GUUGGUGUUUUAUGUUGUUTsT	2204

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Target Pos	Target	Seq ID	Aliases	Sequence	Seq ID
3302	UGACCUUGGAGCAUCUCAUCUGU	2001	KDR:3304U21 siRNA sense	ACCUUGGAGCAUCUCAUCUTT	2044
3852	UUUGAGCAUGGAAGAGGAUUCUG	2002	KDR:3854U21 siRNA sense	UGAGCAUGGAAGAGGAUUCTT	2045
3892	UCACCUGUUUCCUGUAUGGAGGA	2003	KDR:3894U21 siRNA sense	ACCUGUUUCCUGUAUGGAGTT	2046
3946	GACAACACAGCAGGAAUCAGUCA	2004	KDR:3948U21 siRNA sense	CAACACAGCAGGAAUCAGUTT	2047
3302	UGACCUUGGAGCAUCUCAUCUGU	2001	KDR:3322L21 siRNA (3304C) antisense	AGAUGAGAUGCUCCAAGGUTT	2048
3852	UUUGAGCAUGGAAGAGGAUUCUG	2002	KDR:3872L21 siRNA (3854C) antisense	GAAUCCUCUUCCAUGCUCATT	2049
3892	UCACCUGUUUCCUGUAUGGAGGA	2003	KDR:3912L21 siRNA (3894C) antisense	CUCCAUACAGGAAACAGGUTT	2050
3946	GACAACACAGCAGGAAUCAGUCA	2004	KDR:3966L21 siRNA (3948C) antisense	ACUGAUUCCUGCUGUGUUGTT	2051
3302	UGACCUUGGAGCAUCUCAUCUGU	2001	KDR:3304U21 siRNA stab04 sense	B AccuuGGAGcAucucAucuTT B	2052
3852	UUUGAGCAUGGAAGAGGAUUCUG	2002	KDR:3854U21 siRNA stab04 sense	B uGAGcAuGGAAGAGGAuucTT B	2053
3892	UCACCUGUUUCCUGUAUGGAGGA	2003	KDR:3894U21 siRNA stab04 sense	B AccuGuuuccuGuAuGGAGTT B	2054
3946	GACAACACAGCAGGAAUCAGUCA	2004	KDR:3948U21 siRNA stab04 sense	B cAAcAcAGcAGGAAucAGuTT B	2055
3302	UGACCUUGGAGCAUCUCAUCUGU	2001	KDR:3322L21 siRNA (3304C) stab05 antisense	AGAuGAGAuGcuccAAGGuTsT	2056
3852	UUUGAGCAUGGAAGAGGAUUCUG	2002	KDR:3872L21 siRNA (3854C) stab05 antisense	GAAuccucuuccAuGcucATsT	2057
3892	UCACCUGUUUCCUGUAUGGAGGA	2003	KDR:3912L21 siRNA (3894C) stab05 antisense	cuccAuAcAGGAAAcAGGuTsT	2058
3946	GACAACACAGCAGGAAUCAGUCA	2004	KDR:3966L21 siRNA (3948C) stab05 antisense	AcuGAuuccuGcuGuGuuGTsT	2059
3302	UGACCUUGGAGCAUCUCAUCUGU	2001	KDR:3304U21 siRNA stab07 sense	B AccuuGGAGcAucucAucuTT B	2060
3852	UUUGAGCAUGGAAGAGGAUUCUG	2002	KDR:3854U21 siRNA stab07 sense	B uGAGcAuGGAAGAGGAuucTT B	2061
3892	UCACCUGUUUCCUGUAUGGAGGA	2003	KDR:3894U21 siRNA stab07 sense	B AccuGuuuccuGuAuGGAGTT B	2062
3946	GACAACACAGCAGGAAUCAGUCA	2004	KDR:3948U21 siRNA stab07 sense	B cAAcAcAGcAGGAAucAGuTT B	2063
3302	UGACCUUGGAGCAUCUCAUCUGU	2001	KDR:3322L21 siRNA (3304C) stab11 antisense	AGAuGAGAuGcuccAAGGuTsT	2064
3852	UUUGAGCAUGGAAGAGGAUUCUG	2002	KDR:3872L21 siRNA (3854C) stab11 antisense	GAAuccucuuccAuGcucATsT	2065
3892	UCACCUGUUUCCUGUAUGGAGGA	2003	KDR:3912L21 siRNA (3894C) stab11 antisense	cuccAuAcAGGAAAcAGGuTsT	2066
3946	GACAACACAGCAGGAAUCAGUCA	2004	KDR:3966L21 siRNA (3948C) stab11 antisense	AcuGAuuccuGcuGuGuuGTsT	2067

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Target	SeqID	RPI#	Alias	Sequence	SeqID
UGUCCACUUACCUGAGGAGCAAG	2017	30785	KDR:3076U21 siRNA stab04 sense	B uccAcuuAccuGAGGAGcATT B	2205
UUUGAGCAUGGAAGAGGAUUCUG	2002	30786	KDR:3854U21 siRNA stab04 sense	B uGAGcAuGGAAGAGGAuucTT B	2053
AUGGUUCUUGCCUCAGAAGAGCU	2018	30787	KDR:4089U21 siRNA stab04 sense	B GGuucuuGccucAGAAGAGTT B	2206
UCUGAAGGCUCAAACCAGACAAG	2019	30788	KDR:4191U21 siRNA stab04 sense	B uGAAGGcucAAAccAGAcATT B	2207
UGUCCACUUACCUGAGGAGCAAG	2017	30789	KDR:3094L21 siRNA (3076C) stab05 antisense	uGcuccucAGGuAAGuGGATsT	2208
UUUGAGCAUGGAAGAGGAUUCUG	2002	30790	KDR:3872L21 siRNA (3854C) stab05 antisense	GAAuccucuuccAuGcucATsT	2057
AUGGUUCUUGCCUCAGAAGAGCU	2018	30791	KDR:4107L21 siRNA (4089C) stab05 antisense	cucuucuGAGGcAAGAAccTsT	2209
UCUGAAGGCUCAAACCAGACAAG	2019	30792	KDR:4209L21 siRNA (4191C) stab05 antisense	uGucuGGuuuGAGccuucATsT	2210
UGUCCACUUACCUGAGGAGCAAG	2017	31426	KDR:3076U21 siRNA sense	UCCACUUACCUGAGGAGCATT	2211
UUUGAGCAUGGAAGAGGAUUCUG	2002	31427	KDR:3854U21 siRNA sense	UGAGCAUGGAAGAGGAUUCTT	2045
AUGGUUCUUGCCUCAGAAGAGCU	2018	31428	KDR:4089U21 siRNA sense	GGUUCUUGCCUCAGAAGAGTT	2212
UCUGAAGGCUCAAACCAGACAAG	2019	31429	KDR:4191U21 siRNA sense	UGAAGGCUCAAACCAGACATT	2213
UGUCCACUUACCUGAGGAGCAAG	2017	31430	KDR:3094L21 siRNA (3076C) antisense	UGCUCCUCAGGUAAGUGGATT	2214
UUUGAGCAUGGAAGAGGAUUCUG	2002	31431	KDR:3872L21 siRNA (3854C) antisense	GAAUCCUCUUCCAUGCUCATT	2049
AUGGUUCUUGCCUCAGAAGAGCU	2018	31432	KDR:4107L21 siRNA (4089C) antisense	CUCUUCUGAGGCAAGAACCTT	2215
UCUGAAGGCUCAAACCAGACAAG	2019	31433	KDR:4209L21 siRNA (4191C) antisense	UGUCUGGUUUGAGCCUUCATT	2216
UGACCUUGGAGCAUCUCAUCUGU	2001	31434	KDR:3304U21 siRNA sense	ACCUUGGAGCAUCUCAUCUTT	2044
UUUGAGCAUGGAAGAGGAUUCUG	2002	31435	KDR:3854U21 siRNA sense	UGAGCAUGGAAGAGGAUUCTT	2045
UCACCUGUUUCCUGUAUGGAGGA	2003	31436	KDR:3894U21 siRNA sense	ACCUGUUUCCUGUAUGGAGTT	2046
GACAACACAGCAGGAAUCAGUCA	2004	31437	KDR:3948U21 siRNA sense	CAACACAGCAGGAAUCAGUTT	2047
UGACCUUGGAGCAUCUCAUCUGU	2001	31438	KDR:3322L21 siRNA (3304C) antisense	AGAUGAGAUGCUCCAAGGUTT	2048
UUUGAGCAUGGAAGAGGAUUCUG	2002	31439	KDR:3872L21 siRNA (3854C) antisense	GAAUCCUCUUCCAUGCUCATT	2049
UCACCUGUUUCCUGUAUGGAGGA	2003	31440	KDR:3912L21 siRNA (3894C) antisense	CUCCAUACAGGAAACAGGUTT	2050
GACAACACAGCAGGAAUCAGUCA	2004	31441	KDR:3966L21 siRNA (3948C) antisense	ACUGAUUCCUGCUGUGUUGTT	2051

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Target Pos	Target	Seq ID	Aliases	Sequence	Seq ID
2009	AGCACUGCCACAAGAAGUACCUG	2005	FLT4:2011U21 siRNA sense	CACUGCCACAAGAAGUACCTT	2068
3919	CUGAAGCAGAGAGAGAGAAGGCA	2006	FLT4:3921U21 siRNA sense	GAAGCAGAGAGAGAGAAGGTT	2069
4036	AAAGAGGAACCAGGAGGACAAGA	2007	FLT4:4038U21 siRNA sense	AGAGGAACCAGGAGGACAATT	2070
4052	GACAAGAGGAGCAUGAAAGUGGA	2008	FLT4:4054U21 siRNA sense	CAAGAGGAGCAUGAAAGUGTT	2071
2009	AGCACUGCCACAAGAAGUACCUG	2005	FLT4:2029L21 siRNA (2011C) antisense	GGUACUUCUUGUGGCAGUGTT	2072
3919	CUGAAGCAGAGAGAGAGAAGGCA	2006	FLT4:3939L21 siRNA (3921C) antisense	CCUUCUCUCUCUCUGCUUCTT	2073
4036	AAAGAGGAACCAGGAGGACAAGA	2007	FLT4:4056L21 siRNA (4038C) antisense	UUGUCCUCCUGGUUCCUCUTT	2074
4052	GACAAGAGGAGCAUGAAAGUGGA	2008	FLT4:4072L21 siRNA (4054C) antisense	CACUUUCAUGCUCCUCUUGTT	2075
2009	AGCACUGCCACAAGAAGUACCUG	2005	FLT4:2011U21 siRNA stab04 sense	B cAcuGccAcAAGAAGuAccTT B	2076
3919	CUGAAGCAGAGAGAGAGAAGGCA	2006	FLT4:3921U21 siRNA stab04 sense	B GAAGcAGAGAGAGAGAAGGTT B	2077
4036	AAAGAGGAACCAGGAGGACAAGA	2007	FLT4:4038U21 siRNA stab04 sense	B AGAGGAAccAGGAGGAcAATT B	2078
4052	GACAAGAGGAGCAUGAAAGUGGA	2008	FLT4:4054U21 siRNA stab04 sense	B cAAGAGGAGcAuGAAAGuGTT B	2079
2009	AGCACUGCCACAAGAAGUACCUG	2005	FLT4:2029L21 siRNA (2011C) stab05 antisense	GGuAcuucuuGuGGcAGuGTsT	2080
3919	CUGAAGCAGAGAGAGAGAAGGCA	2006	FLT4:3939L21 siRNA (3921C) stab05 antisense	ccuucucucucucuGcuucTsT	2081
4036	AAAGAGGAACCAGGAGGACAAGA	2007	FLT4:4056L21 siRNA (4038C) stab05 antisense	uuGuccuccuGGuuccucuTsT	2082
4052	GACAAGAGGAGCAUGAAAGUGGA	2008	FLT4:4072L21 siRNA (4054C) stab05 antisense	cAcuuucAuGcuccucuuGTsT	2083
2009	AGCACUGCCACAAGAAGUACCUG	2005	FLT4:2011U21 siRNA stab07 sense	B *cAcuGccAcAAGAAGuAcc*TT B	2084
3919	CUGAAGCAGAGAGAGAGAAGGCA	2006	FLT4:3921U21 siRNA stab07 sense	B *GAAGcAGAGAGAGAGAAGG*TT B	2085
4036	AAAGAGGAACCAGGAGGACAAGA	2007	FLT4:4038U21 siRNA stab07 sense	B *AGAGGAAccAGGAGGAcAA*TT B	2086
4052	GACAAGAGGAGCAUGAAAGUGGA	2008	FLT4:4054U21 siRNA stab07 sense	B *cAAGAGGAGcAuGAAAGuG*TT B	2087
2009	AGCACUGCCACAAGAAGUACCUG	2005	FLT4:2029L21 siRNA (2011C) stab11 antisense	*GGuAcuucuuGuGGcAGuG*TsT	2088
3919	CUGAAGCAGAGAGAGAGAAGGCA	2006	FLT4:3939L21 siRNA (3921C) stab11 antisense	ccuucucucucucu*G*cuucTsT	2089

4036	AAAGAGGAACCAGGAGGACAAGA	2007	FLT4:4056L21 siRNA (4038C) stab11 antisense	uuGuccuccuGGuuccucuTsT	2090
4052	GACAAGAGGAGCAUGAAAGUGGA	2008	FLT4:4072L21 siRNA (4054C) stab11 antisense	cAcuuucAuGcuccucuuGTsT	2091

Uppercase = ribonucleotide
u,c = 2'-deoxy-2'-fluoro U,C
T = thymidine
B = inverted deoxy abasic
s = phosphorothioate linkage
A = deoxy Adenosine
G = deoxy Guanosine

Table IV

Non-limiting examples of Stabilization Chemistries for chemically modified siNA constructs

Chemistry	pyrimidine	Purine	cap	p=S	Strand
"Stab 1"	Ribo	Ribo	-	5 at 5'-end 1 at 3'-end	S/AS
"Stab 2"	Ribo	Ribo	-	All linkages	Usually AS
"Stab 3"	2'-fluoro	Ribo	-	4 at 5'-end 4 at 3'-end	Usually S
"Stab 4"	2'-fluoro	Ribo	5' and 3'-ends	-	Usually S
"Stab 5"	2'-fluoro	Ribo	-	1 at 3'-end	Usually AS
"Stab 6"	2'-O-Methyl	Ribo	5' and 3'-ends	-	Usually S
"Stab 7"	2'-fluoro	2'-deoxy	5' and 3'-ends	-	Usually S
"Stab 8"	2'-fluoro	2'-O-Methyl	-	1 at 3'-end	Usually AS
"Stab 9"	Ribo	Ribo	5' and 3'-ends	-	Usually S
"Stab 10"	Ribo	Ribo	-	1 at 3'-end	Usually AS
"Stab 11"	2'-fluoro	2'-deoxy	-	1 at 3'-end	Usually AS

CAP = any terminal cap, see for example **Figure 10.**

All Stab 1-11 chemistries can comprise 3'-terminal thymidine (TT) residues

All Stab 1-11 chemistries typically comprise 21 nucleotides, but can vary as described herein.

S = sense strand

AS = antisense strand

Table V

A. 2.5 µmol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	6.5	163 µL	45 sec	2.5 min	7.5 min
S-Ethyl Tetrazole	23.8	238 µL	45 sec	2.5 min	7.5 min
Acetic Anhydride	100	233 µL	5 sec	5 sec	5 sec
N-Methyl Imidazole	186	233 µL	5 sec	5 sec	5 sec
TCA	176	2.3 mL	21 sec	21 sec	21 sec
Iodine	11.2	1.7 mL	45 sec	45 sec	45 sec
Beaucage	12.9	645 µL	100 sec	300 sec	300 sec
Acetonitrile	NA	6.67 mL	NA	NA	NA

B. 0.2 µmol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	15	31 µL	45 sec	233 sec	465 sec
S-Ethyl Tetrazole	38.7	31 µL	45 sec	233 min	465 sec
Acetic Anhydride	655	124 µL	5 sec	5 sec	5 sec
N-Methyl Imidazole	1245	124 µL	5 sec	5 sec	5 sec
TCA	700	732 µL	10 sec	10 sec	10 sec
Iodine	20.6	244 µL	15 sec	15 sec	15 sec
Beaucage	7.7	232 µL	100 sec	300 sec	300 sec
Acetonitrile	NA	2.64 mL	NA	NA	NA

C. 0.2 µmol Synthesis Cycle 96 well Instrument

Reagent	Equivalents:DNA/ 2'-O-methyl/Ribo	Amount: DNA/2'-O-methyl/Ribo	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time* Ribo
Phosphoramidites	22/33/66	40/60/120 µL	60 sec	180 sec	360sec
S-Ethyl Tetrazole	70/105/210	40/60/120 µL	60 sec	180 min	360 sec
Acetic Anhydride	265/265/265	50/50/50 µL	10 sec	10 sec	10 sec
N-Methyl Imidazole	502/502/502	50/50/50 µL	10 sec	10 sec	10 sec
TCA	238/475/475	250/500/500 µL	15 sec	15 sec	15 sec
Iodine	6.8/6.8/6.8	80/80/80 µL	30 sec	30 sec	30 sec
Beaucage	34/51/51	80/120/120	100 sec	200 sec	200 sec
Acetonitrile	NA	1150/1150/1150 µL	NA	NA	NA

- Wait time does not include contact time during delivery.
- Tandem synthesis utilizes double coupling of linker molecule

CLAIMS

What we claim is:

1. A double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a vascular endothelial growth factor receptor (VEGFr) gene, wherein said siNA molecule comprises about 21 nucleotides.

2. The siNA molecule of claim 1, wherein said siNA molecule comprises no ribonucleotides.

3. The siNA molecule of claim 1, wherein said siNA molecule comprises ribonucleotides.

4. The siNA molecule of claim 1, wherein one of the strands of said double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of a VEGFr gene, and wherein the second strand of said double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of said VEGFr gene.

5. The siNA molecule of claim 4, wherein each said strand of the siNA molecule comprises about 19 to about 23 nucleotides, and wherein each said strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand.

6. The siNA molecule of claim 1, wherein said siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of a VEGFr gene, and wherein said siNA further comprises a sense region, wherein said sense region comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of said VEGFr gene.

7. The siNA molecule of claim 6, wherein said antisense region and said sense region each comprise about 19 to about 23 nucleotides, and wherein said antisense region comprises at least about 19 nucleotides that are complementary to nucleotides of the sense region.

8. The siNA molecule of claim 1, wherein said siNA molecule comprises a sense region and an antisense region and wherein said antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of RNA

encoded by a VEGFr gene and said sense region comprises a nucleotide sequence that is complementary to said antisense region.

9. The siNA molecule of claim 6, wherein said siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of said siNA molecule.

10. The siNA molecule of claim claim 6, wherein said sense region is connected to the antisense region via a linker molecule.

11. The siNA molecule of claim 10, wherein said linker molecule is a polynucleotide linker.

12. The siNA molecule of claim 10, wherein said linker molecule is a non-nucleotide linker.

13. The siNA molecule of claim 6, wherein pyrimidine nucleotides in the sense region are 2'-O-methyl pyrimidine nucleotides.

14. The siNA molecule of claim 6, wherein purine nucleotides in the sense region are 2'-deoxy purine nucleotides.

15. The siNA molecule of claim 6, wherein the pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides.

16. The siNA molecule of claim 9, wherein the fragment comprising said sense region includes a terminal cap moiety at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the fragment comprising said sense region.

17. The siNA molecule of claim 16, wherein said terminal cap moiety is an inverted deoxy abasic moiety.

18. The siNA molecule of claim 6, wherein the pyrimidine nucleotides of said antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides

19. The siNA molecule of claim 6, wherein the the purine nucleotides of said antisense region are 2'-O-methyl purine nucleotides.

20. The siNA molecule of claim 6, wherein the purine nucleotides present in said antisense region comprise 2'-deoxy- purine nucleotides.

21. The siNA molecule of claim 18, wherein said antisense region comprises a phosphorothioate internucleotide linkage at the 3' end of said antisense region.

22. The siNA molecule of claim 6, wherein said antisense region comprises a glyceryl modification at the 3' end of said antisense region.

23. The siNA molecule of claim 9, wherein each of the two fragments of said siNA molecule comprise 21 nucleotides.

24. The siNA molecule of claim 23, wherein about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule and wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule.

25. The siNA molecule of claim 24, wherein each of the two 3' terminal nucleotides of each fragment of the siNA molecule are 2'-deoxy-pyrimidines.

26. The siNA molecule of claim 25, wherein said 2'-deoxy-pyrimidine is 2'-deoxy-thymidine.

27. The siNA molecule of claim 23, wherein all 21 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule.

28. The siNA molecule of claim 23, wherein about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by a VEGFr gene.

29. The siNA molecule of claim 23, wherein 21 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by a VEGFr gene.

30. The siNA molecule of claim 9, wherein the 5'-end of the fragment comprising said antisense region optionally includes a phosphate group.

31. The siNA molecule of claim 1, wherein said VEGFr gene is VEGFr1.

32. The siNA molecule of claim 1, wherein said VEGFr gene is VEGFr2.

33. The siNA molecule of claim 1, wherein said VEGFr gene is VEGFr3.

34. A double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a VEGFr gene, wherein said siNA molecule comprises no

ribonucleotides and wherein each strand of said double-stranded siNA molecule comprisess about 21 nucleotides.

35. The siNA molecule of claim 34, wherein said VEGFr gene is VEGFr1.

36. The siNA molecule of claim 34, wherein said VEGFr gene is VEGFr2.

37. The siNA molecule of claim 34, wherein said VEGFr gene is VEGFr3.

38. A double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a VEGFr gene, wherein said siNA molecule does not require the presence of a ribonucleotide within the siNA molecule for said inhibition of expression of the VEGFr gene and wherein each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

39. The siNA molecule of claim 38, wherein said VEGFr gene is VEGFr1.

40. The siNA molecule of claim 38, wherein said VEGFr gene is VEGFr2.

41. The siNA molecule of claim 38, wherein said VEGFr gene is VEGFr3.

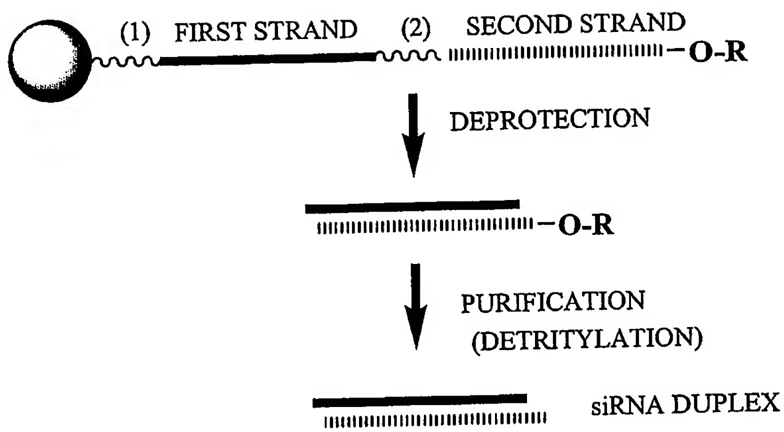
42. A pharmaceutical composition comprising the siNA molecule of claim 1 in an acceptable carrier or diluent.

43. Medicament comprising the siNA molecule of claim 1.

44. Active ingredient comprising the siNA molecule of claim 1.

45. Use of a double-stranded short interfering nucleic acid (siNA) molecule to down-regulate expression of a VEGFr gene, wherein said siNA molecule comprises one or more chemical modifications and each strand of said double-stranded siNA comprises about 21 nucleotides.

Figure 1

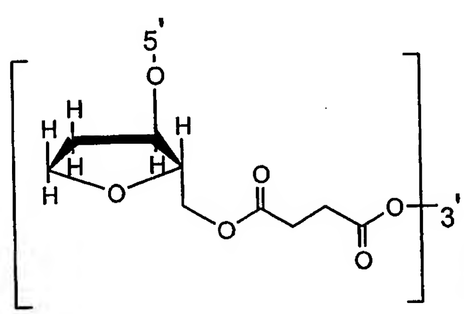


 = SOLID SUPPORT

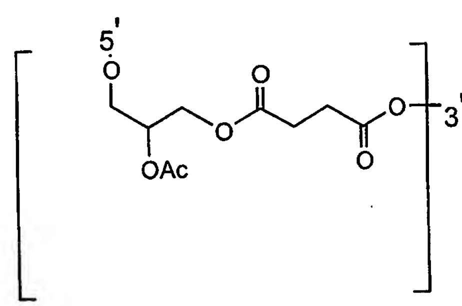
R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

(1) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR
INVERTED DEOXYABASIC SUCCINATE)

(2) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR
INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE LINKAGE



GLYCERYL SUCCINATE LINKAGE

Figure 2

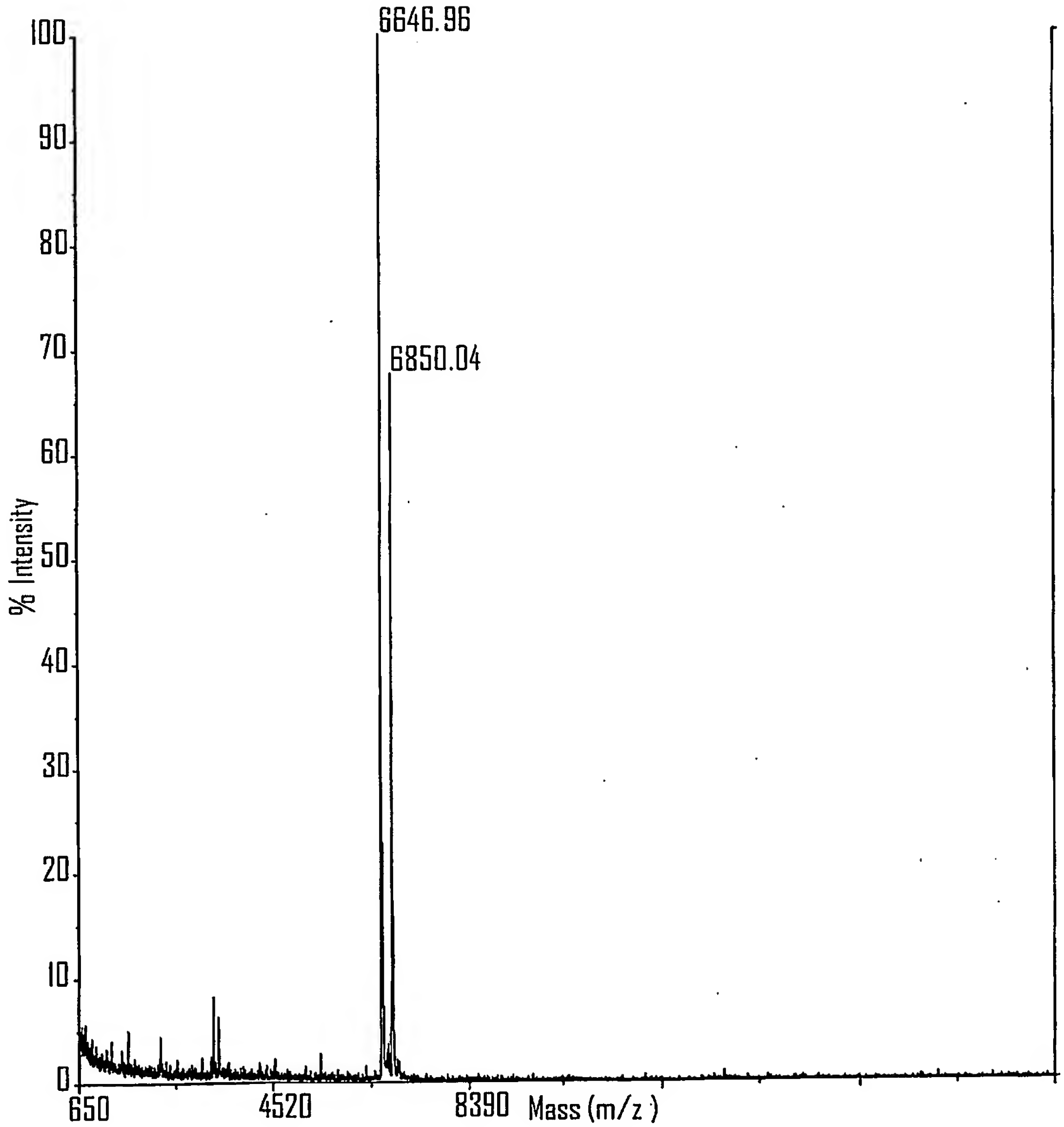

6646.96
6850.04
100
90
80
70
60
50
40
30
20
10
0
% Intensity
650
4520
8390
Mass (m/z)

Figure 3

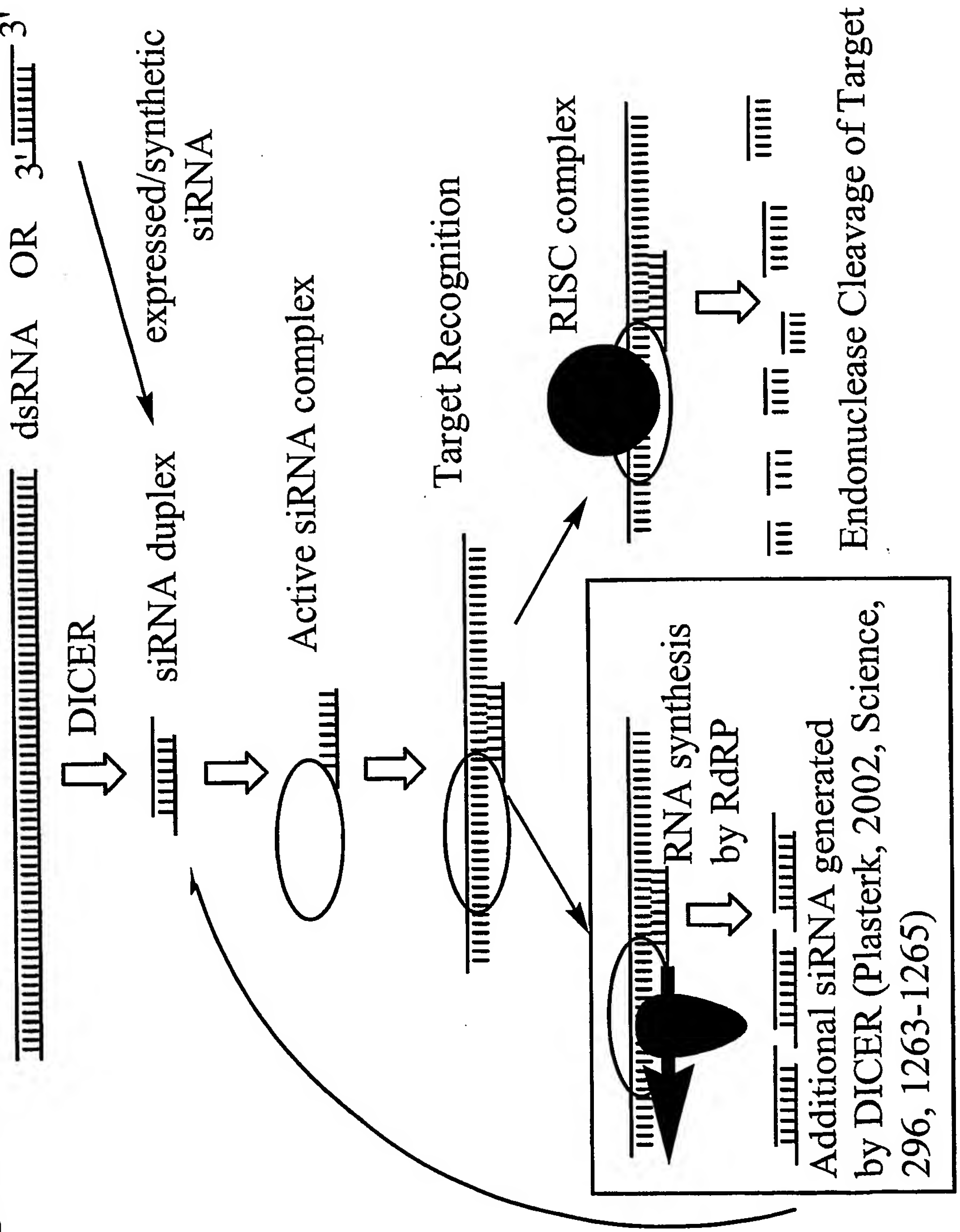

dsRNA OR 3' 3'
DICER
siRNA duplex
expressed/synthetic siRNA
Active siRNA complex
Target Recognition
RISC complex
Endonuclease Cleavage of Target
RNA synthesis by RdRP
Additional siRNA generated by DICER (Plasterk, 2002, Science, 296, 1263-1265)

Figure 4

A

SENSE STRAND (SEQ ID NO 2217)
ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)

5'- $N_s N_s N_s N_s$ N N N N N N N N N N N N $N_s N_s N_s (N_s N)$ -3'
3'- L-$(N_s N)$ N N N N N N N N N N N N N N $N_s N_s N_s N_s$ N -5'

ANTISENSE STRAND (SEQ ID NO 2218)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

B

SENSE STRAND (SEQ ID NO 2219)
ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)

5'- N N N N N N N N N N N N N N N N N N N (N N) -3'
3'- L-(N N) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2220)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

C

SENSE STRAND (SEQ ID NO 2221)
ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-$(N_s N)$ N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2222)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

D

SENSE STRAND (SEQ ID NO 2223)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-$(N_s N)$ N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2224)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

E

SENSE STRAND (SEQ ID NO 2225)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N N) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2226)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

F

SENSE STRAND (SEQ ID NO 2223)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-$(N_s N)$ N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 2227)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES
B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT
L = GLYCERYL MOIETY THAT IS OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE

Figure 5

A

SENSE STRAND (SEQ ID NO 2228)

5'- $c_S A_S A_S c_S$ c A c A A A A u A c A A $c_S A_S A_S T_S T$ -3'

3'- L-$T_S T$ G *u u* G G *u* G *u u u u* A *u* G $u_S u_S G_S u_S u$ -5'

ANTISENSE STRAND (SEQ ID NO 2229)

B

SENSE STRAND (SEQ ID NO 2230)

5'- c A A c c A c A A A A u A c A A c A A *T T* -3'

3'- L-*T T* G *u u* G G *u* G *u u u u* A *u* G *u u* G *u u* -5'

ANTISENSE STRAND (SEQ ID NO 2231)

C

SENSE STRAND (SEQ ID NO 2232)

5'- iB-c A A c c A c A A A A u A c A A c A A *T T*-iB -3'

3'- L-$T_S T$ G *u u* G G *u* G *u u u u* A *u* G *u u* G *u u* -5'

ANTISENSE STRAND (SEQ ID NO 2233)

D

SENSE STRAND (SEQ ID NO 2234)

5'- iB-*c A A c c A c A A A A u A c A A c A A T T*-iB -3'

3'- L-$T_S T$ *g u u g g u g u u u u* $\underline{a}$ *u g u u g u u* -5'

ANTISENSE STRAND (SEQ ID NO 2235)

E

SENSE STRAND (SEQ ID NO 2236)

5'- iB-*c* A A *c c* A *c* A A A A *u* A *c* A A *c* A A *T T*-iB -3'

3'- L-*T T g u u g g u g u u u u* $\underline{a}$ *u g u u g u u* -5'

ANTISENSE STRAND (SEQ ID NO 2237)

F

SENSE STRAND (SEQ ID NO 2235)

5'- iB-*c A A c c A c A A A A u A c A A c A A T T*-iB -3'

3'- L-$T_S T$ G *u u* G G *u* G *u u u u* A *u* G *u u* G *u u* -5'

ANTISENSE STRAND (SEQ ID NO 2238)

lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro
italic lower case = 2'-deoxy-2'-fluoro
$\underline{\text{underline}}$ = 2'-O-methyl

ITALIC UPPER CASE = DEOXY
B = INVERTED DEOXYABASIC
L = GLYCERYL MOIETY OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE

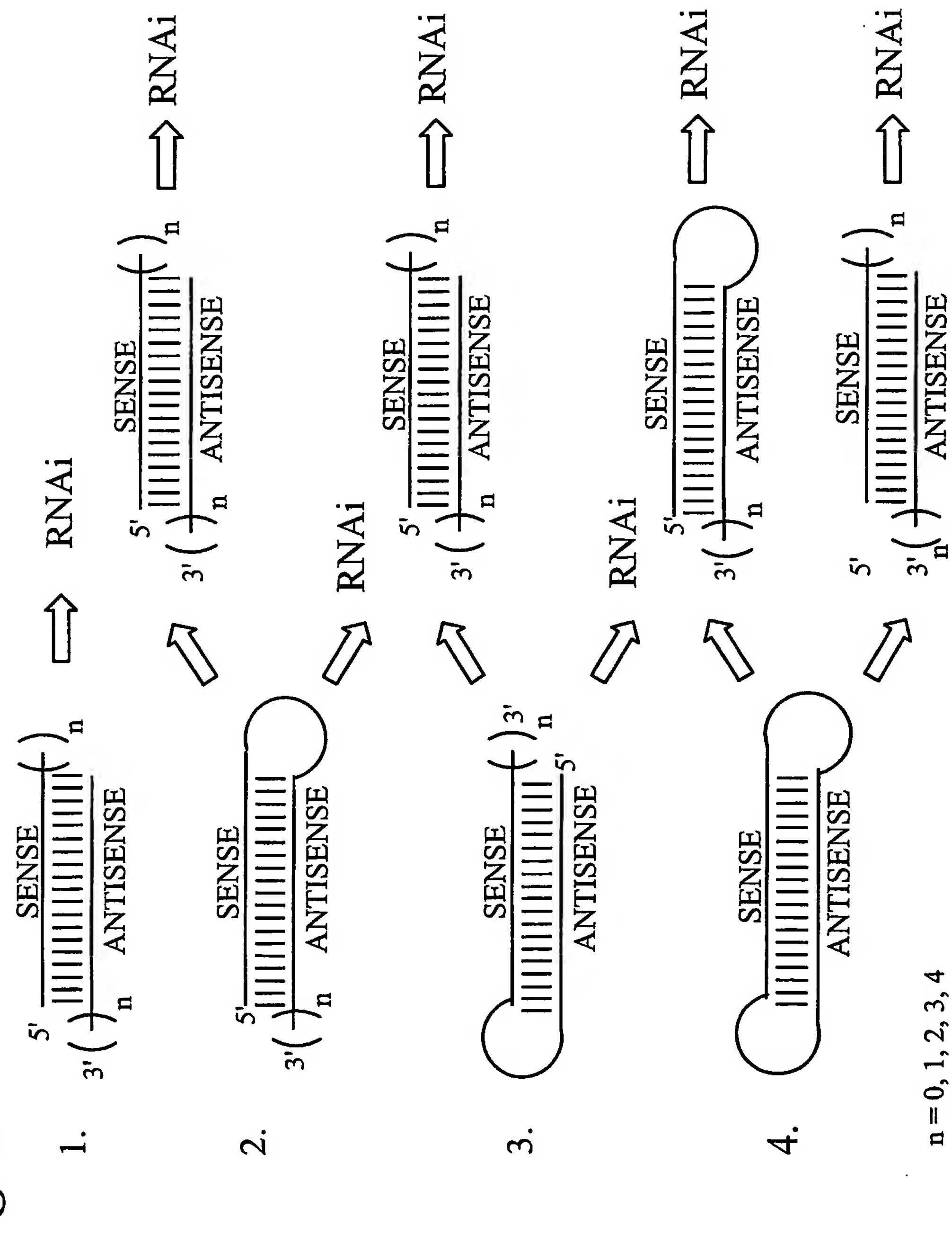


Figure 6

Figure 7

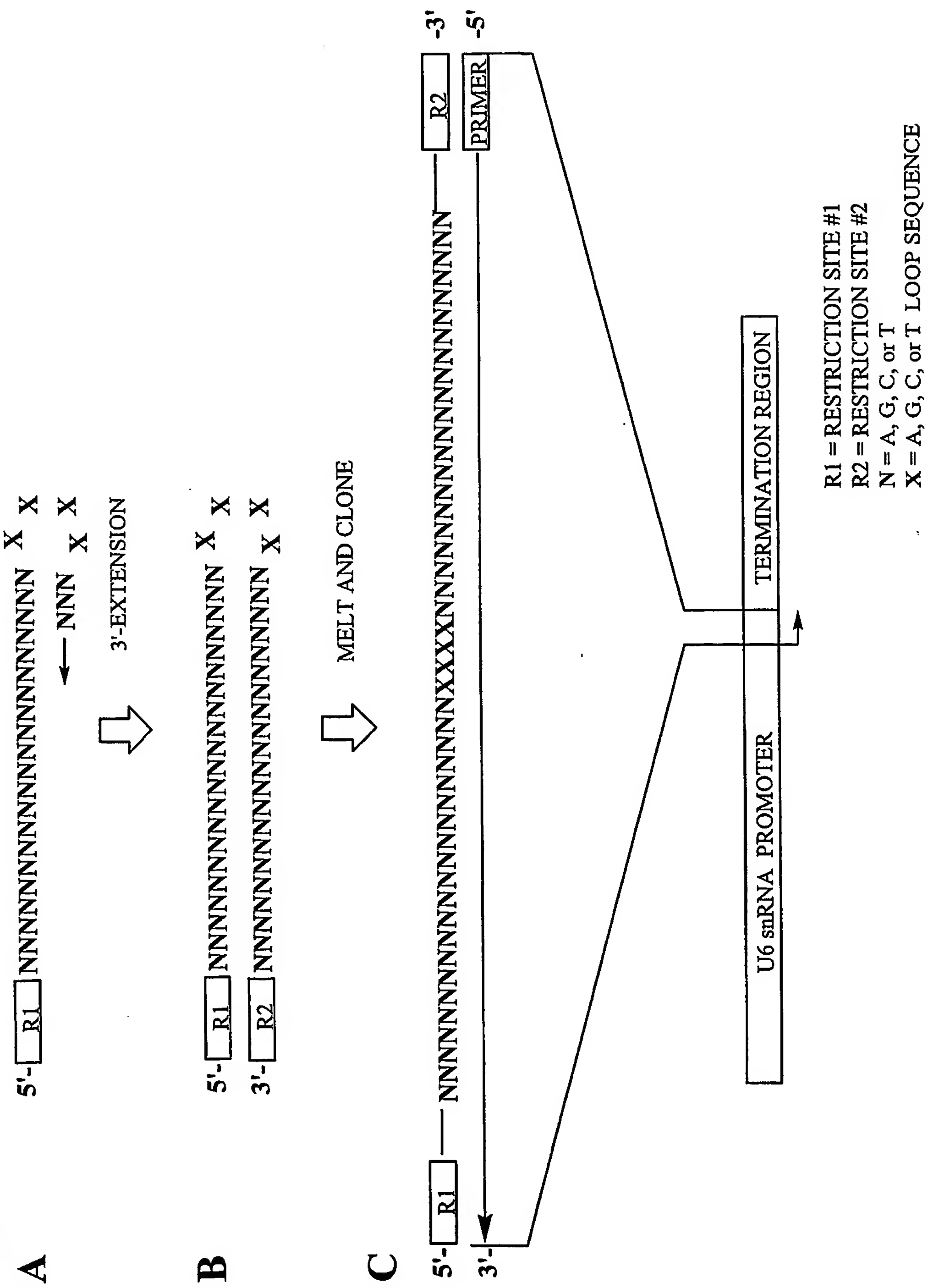

A
5'- R1 NNNNNNNNNNNNNNNNNNNN X X X X
NNN
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNN X X
3'- R2 NNNNNNNNNNNNNNNNNN X X
MELT AND CLONE
C
5'- R1 NNNNNNNNNNNNNNNNNNNXXXXNNNNNNNNNNNNNNNNNNNN R2 -3'
3'- PRIMER -5'
U6 snRNA PROMOTER
TERMINATION REGION
R1 = RESTRICTION SITE #1
R2 = RESTRICTION SITE #2
N = A, G, C, or T
X = A, G, C, or T LOOP SEQUENCE

Figure 8

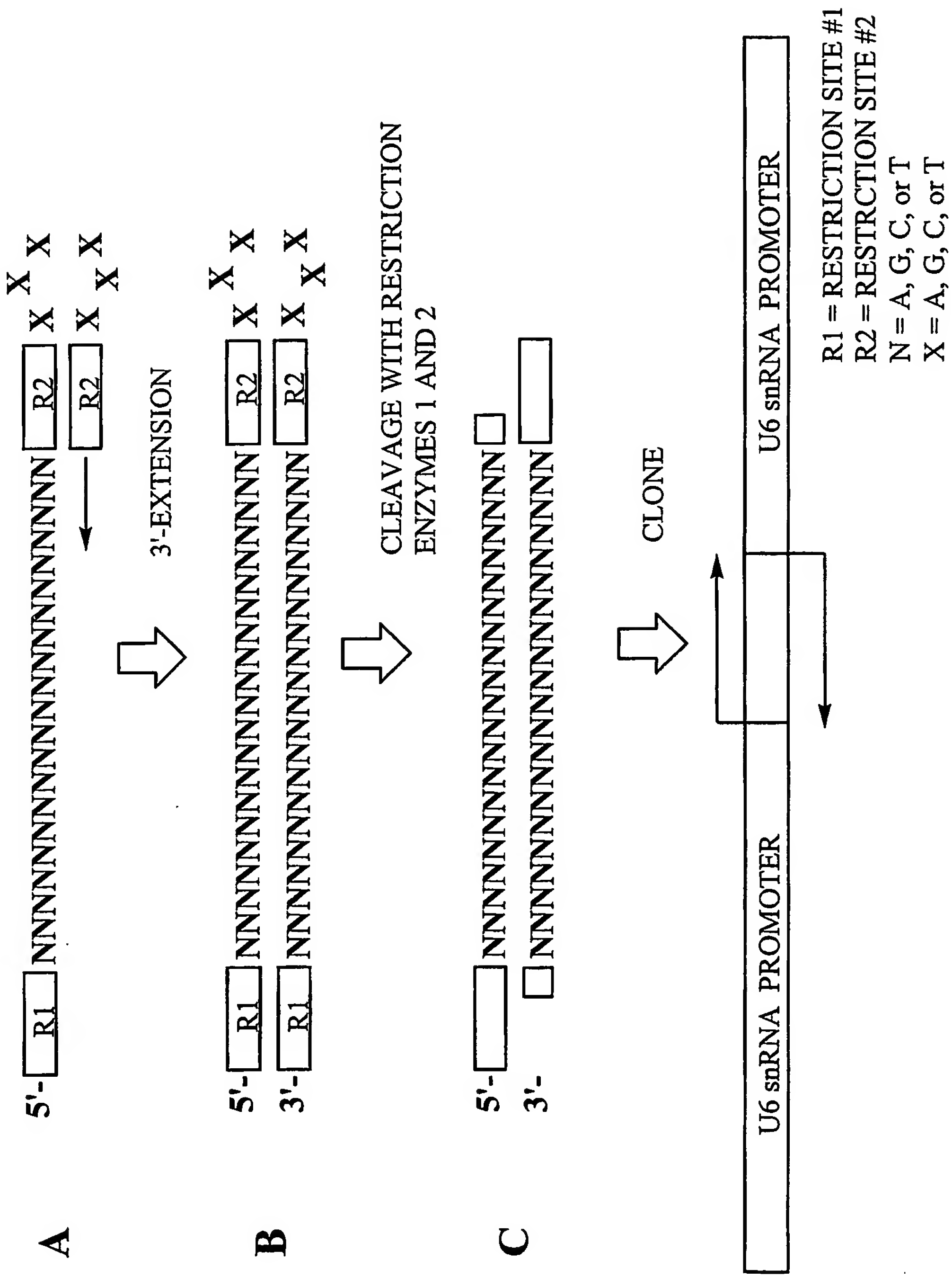

A
5'- R1 NNNNNNNNNNNNNNNNNNNNN R2 XXXXX
R2 XXXX
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNNNN R2 XXXXX
3'- R1 NNNNNNNNNNNNNNNNNNNNN R2 XXXX
CLEAVAGE WITH RESTRICTION ENZYMES 1 AND 2
C
5'- NNNNNNNNNNNNNNNNNNNNN
3'- NNNNNNNNNNNNNNNNNNNNN
CLONE
U6 snRNA PROMOTER
U6 snRNA PROMOTER
R1 = RESTRICTION SITE #1
R2 = RESTRCTION SITE #2
N = A, G, C, or T
X = A, G, C, or T

Figure 9: Target site Selection using siRNA

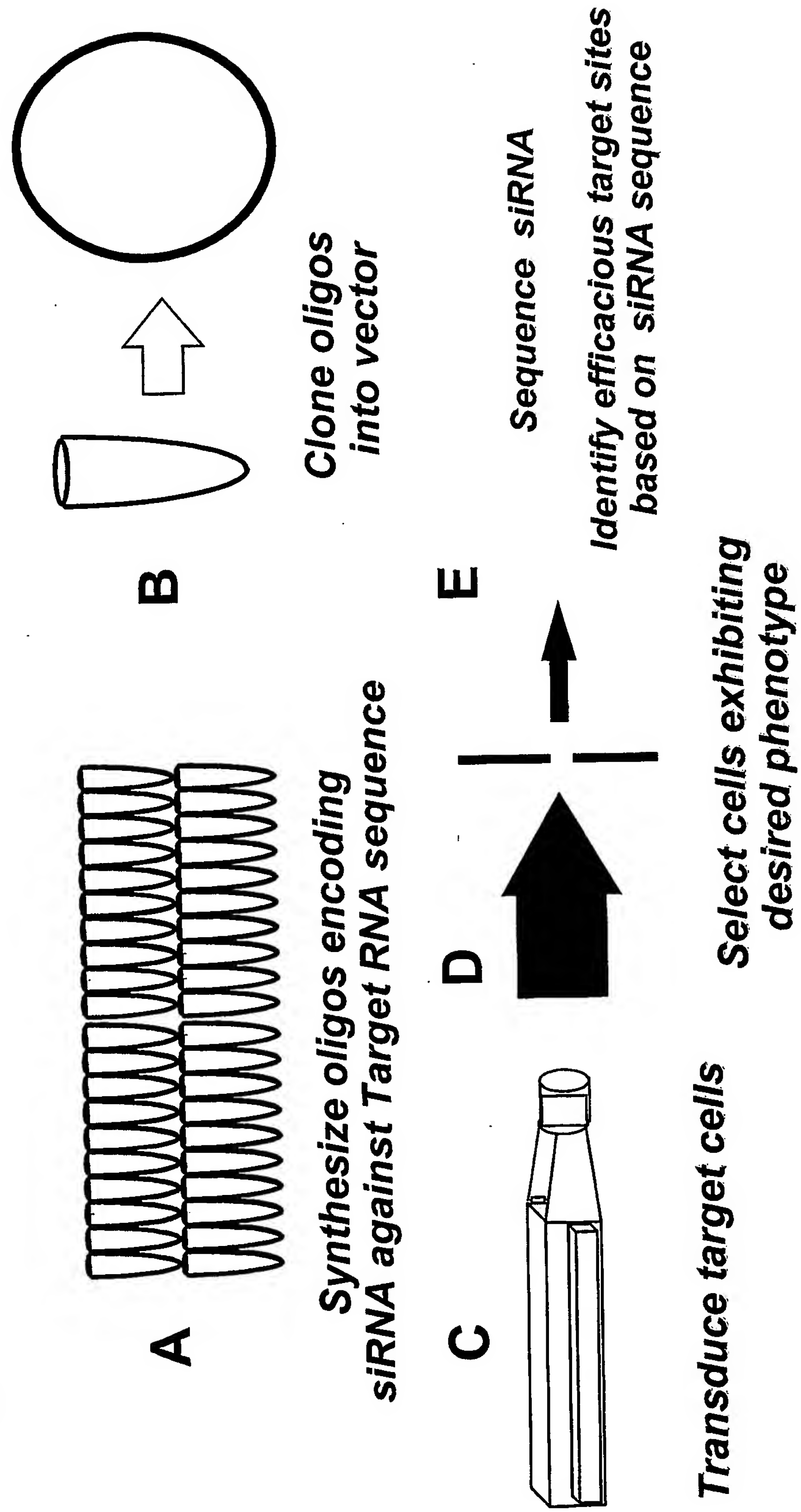
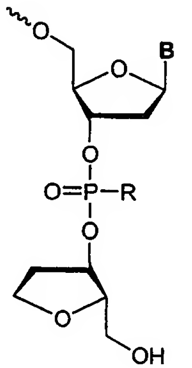


Figure 10



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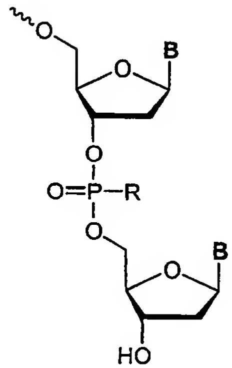

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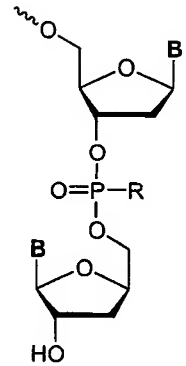

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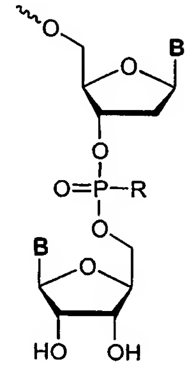

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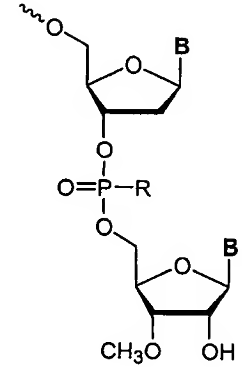

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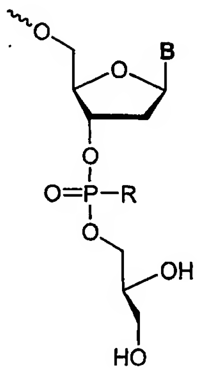

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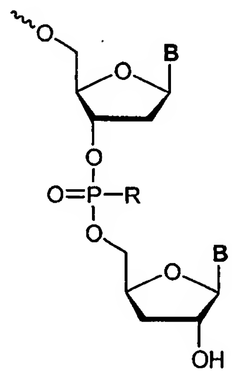

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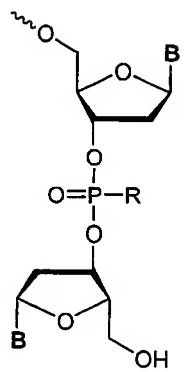

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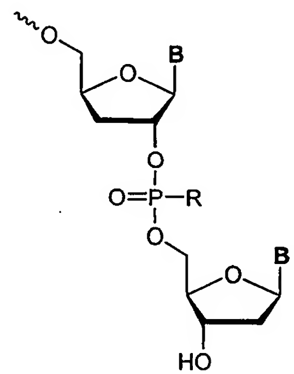

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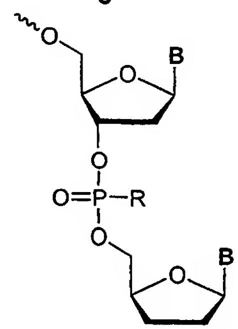

10

R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

Figure 11: Modification Strategy

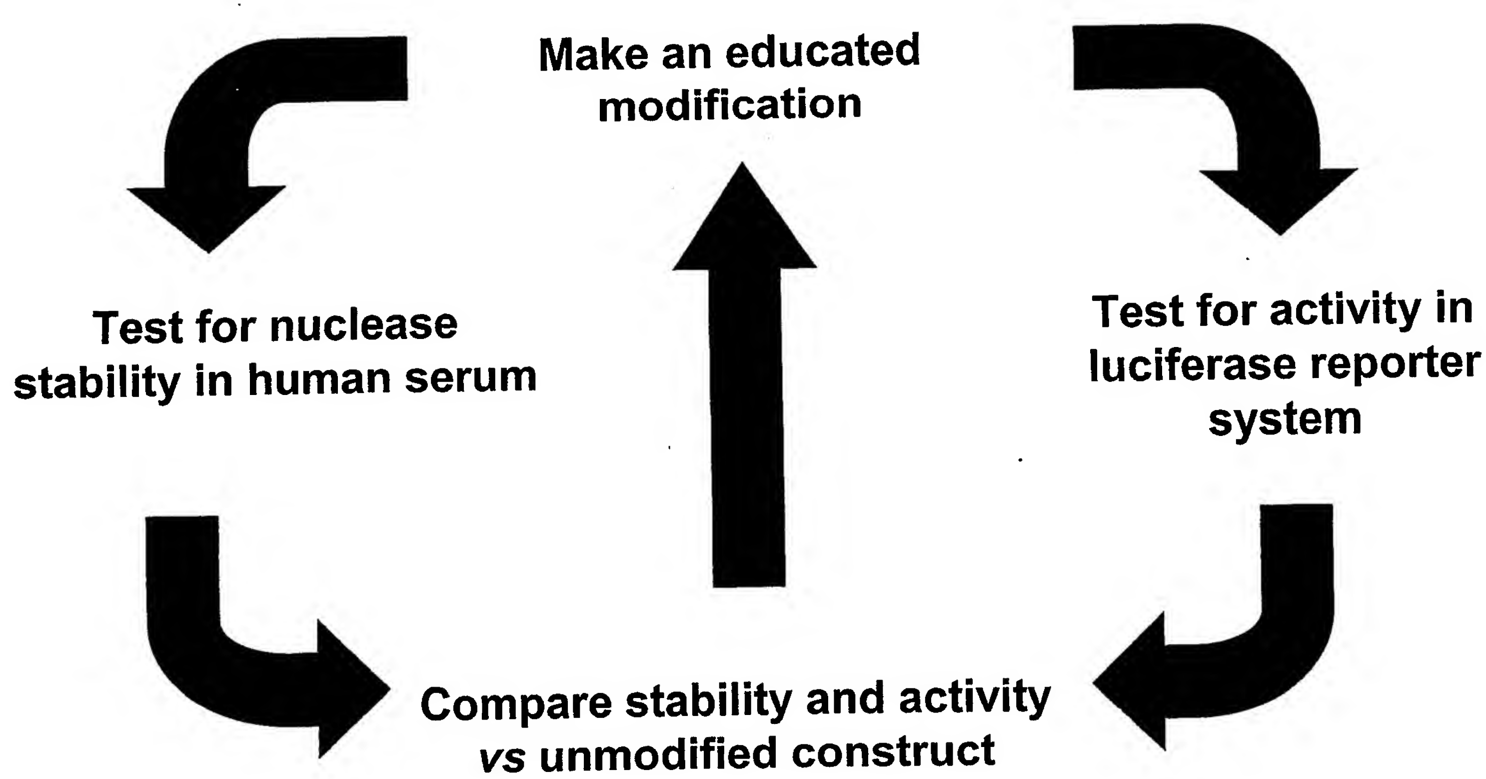

Figure 12: Inhibition of VEGF-Induced Angiogenesis by siRNAs

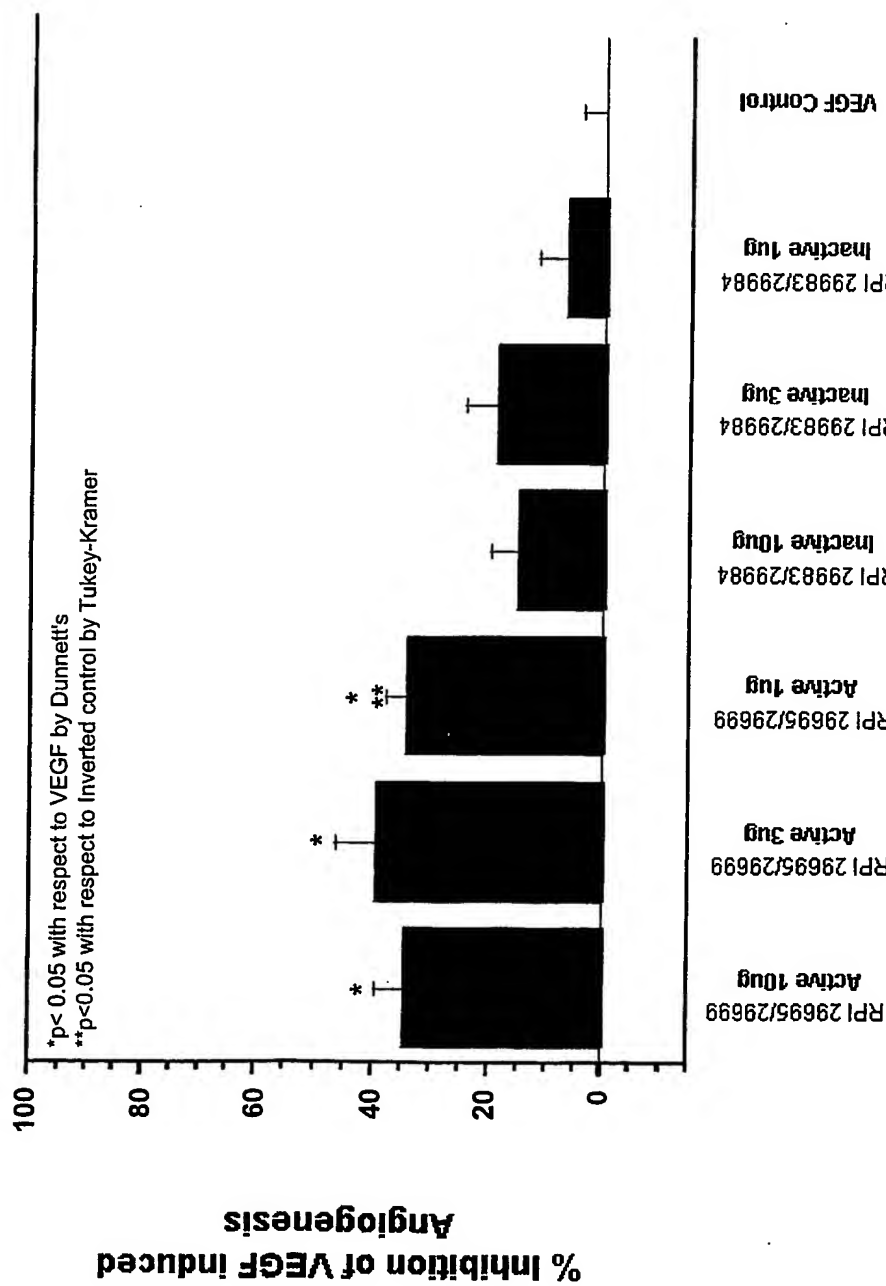

Figure 13: A375 24h 36B4 VEGFR1 mRNA Expression